09/26/90

PEER REVIEW FILES

008080

CHEMICAL NAME: Systane (Myclobutanil/Rally)
CASWELL NO.: 723K
CAS NO.: 88671-89-0
REVIEWER: Hurley

CURRENT AGENCY DECISION

Classification deferred pending submission of repeat rat and mouse studies.

TUMOR TYPE / SPECIES

Repeat of rat (M & F) oncogenicity study using higher dose levels was recommended.; Repeat of mouse (F) oncogenicity study using higher dose levels was recommended.

REVIEWER PEER REVIEW PACKAGE	PEER REVIEW MEETING DATE	PEER REVIEW DOCUMENTS	PEER REVIEW CLASSIFICATION
5. / /	5. / /	5. / /	5.
4. / /	4. / /	4. / /	4.
3. / /	3. / /	3. / /	3.
2. / /	2. / /	2. / /	2.
1. 02/05/88	1. 02/09/88	1. 03/01/88	1. Deferred.

SAP MEETING	SAP CLASSIFICATION
2. / /	2.
1. / /	1.

QUALITATIVE RISK ASSESSMENT DOCUMENT	QUANTITATIVE RISK ASSESSMENT DOCUMENT	GENETIC TOXICITY ASSESSMENT DOCUMENT
3. / /	3. / /	1. / /
2. / /	2. / /	
1. / /	1. / /	

MISCELLANEOUS:

Miscellaneous: 03/21/88 Rally communication from P. K. Chang. Stamped 09/06/90; #PR-008080.

Peer Review Documents
(Memo dates)

3/1/88

008080

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

FILE COPY

MAR 1 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Abbreviated Peer Review Meeting on Rally

FROM: John A. Quest, Ph.D. JAQ 2/25/88
Team Leader, Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Lois Rossi, Product Manager #21
Registration Division (TS-767)

The Toxicology Branch Peer Review Committee met on February 9, 1988, to briefly review the toxicology data base on Rally. The purpose of the meeting was to determine if the doses of the compound selected for testing in long term animal studies were appropriate for evaluating potential oncogenic activity.

A. Individuals in Attendance

1. Peer Review Committee: (Signature indicates concurrence with the conclusions of the peer review unless otherwise stated.)

William Burnam _____

Reto Engler _____

Theodore M. Farber Theodore M. Farber

Judith Hauswirth Judith W. Hauswirth

Richard Levy _____

John A. Quest John A. Quest

Esther Rinde Esther Rinde

2. Scientific Reviewers: (Non-committee members responsible for presentation of data; signature indicates technical accuracy of report.)

Edwin Budd Edwin R. Budd

Pamela Hurley Pamela M. Hurley

3

3. Background:

Rally (RH-3866; (alpha-butyl-alpha-(4-chorophenyl) -1H-1,2,4-triazole-1-propanenitrile) is a new triazole fungicide manufactured by Rohm and Haas, Spring House, PA. The compound has had EUP's with temporary tolerances issued for use on 2 raw commodities, apples and grapes (fresh market only). Other petitions for temporary tolerances on processed commodities of apples and grapes, and meat, milk and eggs are being processed by the Agency. The Peer Review Committee was asked to determine whether a Maximum Tolerated Dose (MTD) was reached in animals of either sex in oncogenicity studies in rats and mice.

4. Oncogenicity Studies and MTD Considerations:

The two oncogenicity studies reviewed consisted of a 2-year rat test and a 2-year mouse test. Ninety day toxicity studies in rats and mice were also evaluated by the Committee. All of the studies were performed by Rohm and Haas, except for the 2-year rat study which was performed by Tegeris Laboratories.

A. Rat Studies:

In the 2-year rat feeding study (doses 50, 200 and 800 ppm), there was no indication of oncogenic effects in either sex. The changes observed were testicular atrophy in males at levels of 200 ppm and above and increased mixed function oxidase activity at levels of 200 ppm and above in females and 800 ppm in males. Increased liver weights were seen in females at 800 ppm at 3 and 6 months and decreased testicular weights were observed in males at 200 ppm and above. There appeared to be sporadic reductions in body weight gain (ranging from -12% to -17% at various intervals in female rats over the last year of the study. These were not considered to be a prominent effect of Rally, however, since they returned to control levels by the very end of the study. None of the above described toxicological findings in rats appeared to be unusually deleterious or life threatening to the animals on the test, suggesting that the doses of Rally selected for testing in this chronic study may not have been high enough to adequately characterize the compound's oncogenic potential.

4

Dose selection for the rat chronic feeding study was based on a 90-day feeding study in the rat (doses 10, 30, 100, 300, 1000, 3000, 10000 and 30000 ppm). In this study, no changes were observed in either sex at doses up to and including 100 ppm. At 300 ppm and above, increases in mixed function oxidase activity in males was observed. At 1000 ppm, accentuated liver architecture was grossly observed in males and females exhibited an increase in relative liver weights as well as increases in mixed function oxidase activity. The report patnologist stated that the NOEL for liver effects was 1000 ppm. At 3000 ppm and above, animals in both sexes exhibited decreases in body weight gain (-17% in males and -13% in females at 90 days and effects in the liver, kidneys, adrenals and thyroid. At 10000 ppm, additional effects were observed in the hematology and clinical chemistry studies.

The registrant estimated the MTD to be 1000 ppm, where moderate toxicity was seen (the Registrant had made an error in calculations here and had originally seen a 20% decrease in body weight gain in females). On the basis of the estimated 1000 ppm MTD, the Registrant selected 800 ppm to be the top dose in the chronic study. The Peer Review Committee, after considering the data from the above described 90-day study, believed that the reductions in body weight gain in males (-17%) and females (-13%) indicated that 3000 ppm was an approximate MTD level in both sexes of rats. As such, the highest dose of Rally tested in males and females in the chronic study (i.e., 800 ppm) was considered to be inadequately low for evaluating potential oncogenic activity.

It should be noted that the Registrant also used the "Decision Tier Scheme to Determine the Need to Repeat Completed Oncogenicity Studies Without MTD Levels", as described in OPP's Draft Position Paper on the MTD (April 10, 1986), to determine that the rat oncogenicity study need not be repeated. Their argument was based on the erroneous information that 1000 ppm in the 90-day study was a MTD, and that 800 ppm in the chronic study was therefore greater than 1/2 MTD. However, as indicated above, the Peer Review Committee believed that 3000 ppm in the 90-day study approximated a MTD level. As such, 800 ppm in the chronic study was not greater than 1/2 MTD, leading the Committee to conclude that the rat study (both sexes) should be repeated.

5

B. Mouse Studies:

In the 2-year mouse feeding study (doses 20, 100 and 500 ppm), there was no indication of oncogenic effects in either sex. The changes observed were increases in liver mixed function oxidase activity (starting at 100 ppm for females and at 500 ppm for males), and increases in liver weights and other liver effects at 500 ppm in both sexes. The liver effects included hepatocellular hypertrophy, Kupffer cell pigmentation, periportal punctate vacuolation, individual cell hepatocellular necrosis, focal hepatocellular alterations and multifocal hepatocellular alterations. The individual cell hepatocellular necrosis did not appear at terminal sacrifice. It was slightly increased in both incidence (6/20), and severity in male mice fed 500 ppm for 12 months when compared to control males (2/20), and characterized by only a few single scattered hepatocytes with pyknotic, karyorrhectic or karyolytic nuclei. A few inflammatory cells, predominantly neutrophils, were occasionally present around or within the necrotic hepatocytes which were predominantly located in centrilobular areas. No significant decrements in body weight gain were observed in either males or females.

Dose selection of the chronic study was based on a 90-day feeding study in the mouse (doses 3, 10, 30, 100, 300, 1000, 3000, and 10000 ppm). In this study, no changes were observed in dose levels up to and including 300 ppm. At 1000 ppm, males had a significant decrease in body weight gain (-37% at 90 days), increases in liver weights and mixed function oxidase activity, hepatocytic hypertrophy, swollen-vacuolated centrilobular hepatocytes and individual hepatocytic necrosis, centrilobular (3/10), as well as cytoplasmic eosinophilia and/or hypertrophy of the zona fasculata cells of the adrenals. Females only had increases in relative liver weights. At 3000 ppm, females began to exhibit the same effects as males, including the decrease in body weight gain (-35% at 90 days).

The Registrant chose the top dose of 500 ppm for the chronic study because he believed that 3000 ppm definitely exceeded the MTD, and that 1000 ppm probably exceeded the MTD, as indicated by the 37% decrease in body weight gain in males and the liver toxicity in both sexes. The Peer Review Committee agreed that 1000 ppm exceeded a MTD in male mice based on the 37% decrease in body weight gain. Therefore, the top dose of 500 ppm in male mice in the chronic study was considered to be $\geq$ than 1/2 MTD

(according to OPP's Decision Tier Scheme) and thus satisfactory for evaluating Rally's oncogenic potential in male mice. In contrast, the Committee believed that 3000 ppm (but not 1000 ppm) exceeded a MTD in female mice based on the 35% decrease in body weight gain. Therefore, the top dose of 500 ppm in female mice in the chronic study was less than an MTD level and thus not adequately high enough to evaluate the compound's oncogenic potential. The dose of 500 ppm was also not $\geq$ than 1/2 MTD. The Committee recommended that the female portion of the mouse oncogenicity study be repeated.

5. Ancillary Toxicology Information:

Other information in addition to oncogenicity and 90-day feeding studies was discussed. Rally was not genotoxic in a battery of mutagenicity assays (rat dominant lethal assay, in vitro cytogenetics in mouse bone marrow, CHO/H GPRT assay, and Ames tests) and was not teratogenic in rabbits or rats. Rally is structurally related to six other triazole/imidazole fungicides (Propiconazole, Triadimefon, Triadimenol, NuStar, Penconazole and Imidazole); positive oncogenicity results were obtained only with two of these chemicals (Propiconazole and Triadimenol) and only in long-term mouse studies. The structure-activity data was considered to be relatively weak. The metabolism of Rally was extensive in the rat (rapidly and completely absorbed from the GI tract with several metabolites formed that are excreted in urine and feces; no bioaccumulation was observed.

6. Conclusions:

The conclusions of the Peer Review Committee concerning MTD issues in the chronic rat and mouse oncogenicity studies of Rally are as follows:

1. Rat Study: There was no indication of oncogenicity in either male or female rats at dose levels ranging from 50 to 800 ppm over a 2 year period. The levels in the chronic study appeared to be inadequate for assessing oncogenic potential. An MTD for male and female rats of about 3000 ppm (for both sexes), could be predicted from a 90-day study, based upon reductions in body weight gain ranging from -13% to -17%. However, this value was not approached in the chronic study. The Committee recommended that a repeat oncogenicity study be performed in male and female rats. The highest dose tested should approach a MTD level (e.g., about 2500 ppm).

7

2. Mouse Study: There was no indication of oncogenicity in either male or female mice at dose levels ranging from 20 to 500 ppm in the chronic study. Based upon the results of the 90-day feeding study in mice, doses of 1000 ppm in males and 3000 ppm in females were predicted to exceed MTD values because of -35% to -37% reductions in body weight gain. Since the top dose of 500 ppm used in male mice in the chronic study was > than 1/2 the predicted MTD value, the Committee considered the male portion of the mouse oncogenicity study to be satisfactory. On the other hand, since the top dose of 500 ppm used in female mice in the chronic study did not approach the predicted MTD value, the Committee considered the female portion of the mouse oncogenicity study to be unsatisfactory and recommended that it be repeated. The highest dose tested should approach a MTD level (e.g., about 2000 ppm).

#24 2/23/88 sp

Reviewer's Peer Review Package for 1st Meeting

2/5/88

008080

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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FEB 5 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review of Dose Selection in Rat and Mouse Study of Ralley.

FROM: Reto Engler, Chief
Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Addressees

Attached for your review is a package prepared by Dr. Pamela Hurley, concerning the selection of appropriate doses for the long-term rat and mouse study of Ralley (new chemical).

A meeting to evaluate the dose selection process for this chemical is scheduled for Monday, February 8, 1988, at 10:00 AM in Dr. Farber's office.

Attachment

ADDRESSEES:

T. Farber
R. Levy
J. Quest
E. Rinde
W. Burnam
J. Hauswirth
E. Budd
P. Hurley
L. Rossi (PM #21)

#23 2/5/88:sp

10

008080

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

FEB 4 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: MTD Issue In Rat and Mouse Oncogenicity Studies on RH-3866

FROM: Pamela M. Hurley, Toxicologist
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

TO: Reto Engler, Chief
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769c)

The attached data evaluation report is in semi-Peer review format. It is requested that a Branch Committee review the package and discuss the issues presented.

Attachments

TOXICOLOGY SUMMARY FOR BRANCH REVIEW OF RH-3866

Summary Of Issue

Appendices

Summary of Issue

Two oncogenicity studies have been conducted on the new fungicide, RH-3866. The review committee is asked to evaluate whether or not the Maximum Tolerated Dose (MTD) was approached in either sex in the two studies. If it was not approached, the committee is asked to recommend repetition of the study(ies), if necessary in order to satisfy the EPA Testing Guidelines (83-3).

I. Background

RH-3866 (alpha-butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile) (see Figure 1) is a new triazole fungicide. EUP's and temporary tolerances have been issued for use for the raw commodities, apples and grapes (fresh market only). Other petitions for temporary tolerances on processed commodities of apples and grapes, and meat, milk and eggs are being processed by the Agency.

This chemical has passed the new chemical screen and is in the process of being evaluated for full registration.

II. Metabolism of RH-3866

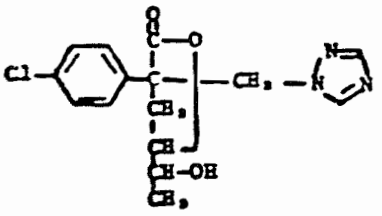
- o Following oral administration, RH-3866 is completely and rapidly absorbed from the G.I. tract in rats.
- o It is extensively metabolized, and rapidly and essentially completely excreted.
- o The eliminated dose is evenly divided between urine and feces.
- o No tissue accumulation was observed after 96 hours.
- o Pretreatment for 2 weeks with nonlabeled material had little effect on the disposition and metabolism of a single oral dose.
- o 7 major metabolites were recovered and specifically identified. Their structures are as follows:

Figure 1

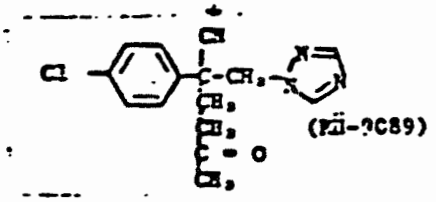
Structures of Metabolites

Structure

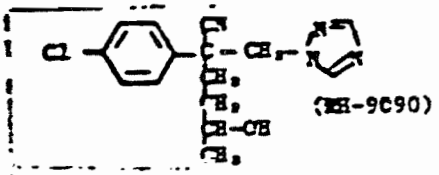
RH-3866



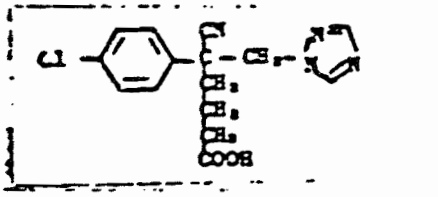
Another Isomer of (2)

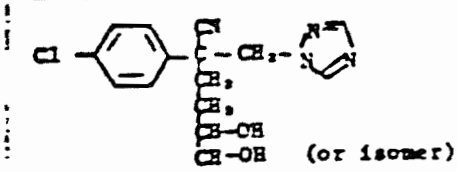


(RH-9089)

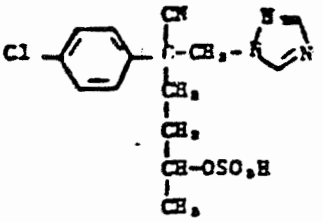


(RH-9090)





(or isomer)

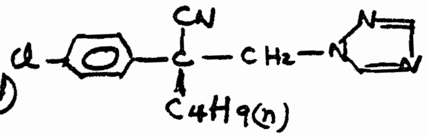
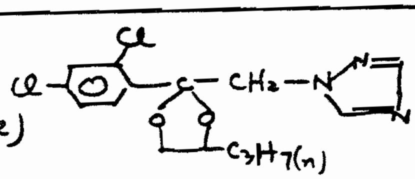
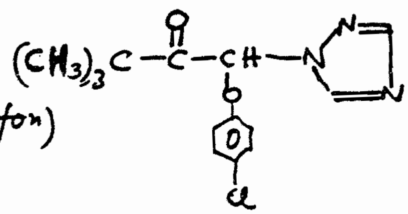
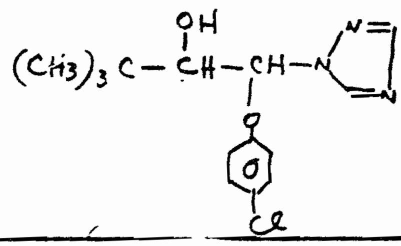
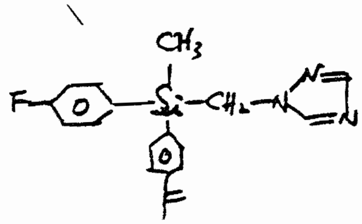
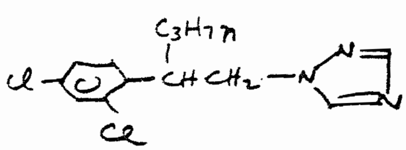
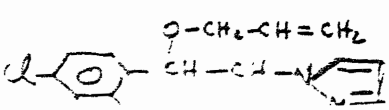


III. Structure Activity Relationships

The Registrant presented a comparison of the oncogenicity study data of RH-3866 with data from other analogues of triazole/imidazole fungicides. Figure 2 presents the comparison data.

Figure 2

Analogues of Triazole/Imidazole Fungicides

Name	Structure	Carcinogenicity (HDT) 2-yr Rat	Carcinogenicity (HDT) 2-yr Mouse
RH-3866 (Mycobutanil)		Negative (800 ppm) NOEL = 50 ppm. Testicular Atrophy at ≥200 ppm	Negative (500 NOEL = 20 pp Liver toxicity ≥ 100 ppm
Tilt (Propiconazole)		Negative (5000 ppm) NOEL = 100 ppm	positive (2500 p Negative at low doses NOEL = 100 p.
Bayleton (Triadimefon)		Negative (500 ppm) HDT 5000 ppm Terminated at 39 wk — toxic NOEL = ?	Negative (180 NOEL = ?
Baytan (Triadimenol)		Negative NOEL = ?	positive (F) NOEL = ?
Nustar ( )		Negative (250 ppm) NOEL = 10 ppm MTD not reached	Negative 200 ppm NOEL = 25 ppm MTD not reached
Topas (Penconazole)		?	?
Imazalil (Imidazole)		Negative (400 ppm)	Negative ([illegible]) in drinking water

IV. Relevant Subchronic and Oncogenicity Studies

A. Mouse

[See Tables 1-6 for comparative summary of toxicity and Appendix I for DERs of these studies].

1. 90-Day Feeding Study - Mouse (Core Grade Guideline)

Strain: Crl:CD®-1(ICR)BR
Age: 8 weeks
Weight: 32-34 g (males), 24-26 g (females)
Source: Charles River Breeding Labs, Stone Ridge, N.Y.
Testing Facility: Rohm & Haas Toxicology Dept.
Spring House, PA
Total mice tested in study: 180

Nine groups of ten mice of each sex were fed different dose levels of RH-3866 daily in the diet for 13 weeks. The following dose levels were administered: 0, 3, 10, 30, 100, 300, 1000, 3000 and 10000 ppm.

The NOEL for males is 300 ppm and the NOEL for females is 1000 ppm based upon decreased body weight gain, hepatocytic hypertrophy, swollen vacuolated hepatocytes, individual hepatocytic necrosis (not evident at highest dose level), increased liver weights and eosinophilia and/or hypertrophy of the zona fasculata cells of the adrenal glands. See Tables 1 and 2 for details.

There was no treatment-related effect on survival and the only clinical sign of toxicity was scant fecal droppings in the highest dose group. Decreases in body weight gains were observed in males starting 1000 ppm and in females starting at 3000 ppm. Significant decreases in mean body weights were only observed at 10000 ppm in both sexes. See Tables 3 and 4 for details on mean body weights and mean body weight gain as well as individual animal data on body weight gains for males at 1000 ppm. Decreases in food consumption were observed at the 10000 ppm dose level in both sexes, particularly during the first week of treatment. Hematological changes were noted at the highest dose level and changes in clinical chemistry values were noted in males starting at 1000 ppm and in females starting at 3000 ppm. Liver weights were increased in both sexes starting at 1000 ppm. Increases in liver mixed function oxidase activity were observed in males starting at 1000 ppm and in females starting at 3000 ppm.

2. 2-Year Chronic Feeding/Oncogenicity Study - Mouse
(Core Grades: Chronic - Guideline; Oncogenicity - Reserved)

Strain: Crl:CD®-1(ICR)BR mice
Age: 3 weeks upon receipt
Weight: Not given
Source: Charles River Breeding Labs
Testing Facility: Toxicology Dept., Rohm & Haas Company, Spring House, PA
Total mice tested in study: 70 mice/sex/group (4 groups), 10/sex/group for interim sacrifices at 3 and 6 months, 20/sex/group for interim sacrifice at 12 months, and 25 male and 25 female sentinels.

Mice were treated up to 24 months with the following dose levels: 0, 20, 100 and 500 ppm.

No oncogenic effects were observed.

Maximum Tolerated Dose (MTD) - This issue is reserved pending the decision made by the review committee following the review of this study and the 90-day study.

The NOEL for increased mixed function oxidase in the liver in the 2-year chronic feeding study in mice is 20 ppm in females and 100 ppm in males. The LEL is 100 ppm for females and 500 ppm for males. The NOEL for liver effects is 100 ppm in both sexes. The LEL is 500 ppm (HDT), based upon hepatocellular hypertrophy, Kupffer cell pigmentation, periportal punctate vacuolation, individual hepatocellular necrosis (not present at terminal sacrifice), focal hepatocellular alterations (only present at terminal sacrifice) and multifocal hepatocellular alterations (only present at terminal sacrifice). See Table 5 for details.

There were no treatment-related effects on survival, nor were there any clinical signs of toxicity. A slight effect on body weight gain in females at 500 ppm may have been present, but the effect was not consistent. No significant differences in body weights were noted for either sex. No differences in food consumption were observed and no treatment-related changes in hematological values were observed. An increase in SGPT was observed in females at 500 ppm after 3 months of treatment, but not at any other time. No other clinical chemistry changes were observed. Increases in liver weights were observed in both sexes at 3 months. See Tables 1 and 5 for details.

TABLE I

Comparative Toxicity

MICE

	MALE		FEMALE	
PPM	90-DAY	2-YEAR	90-DAY	2-YEAR
3	NC	--------	NC	--------
10	NC	--------	NC	--------
20	--------	NC	--------	NC
30	NC	--------	NC	--------
100	NC	NC	NC	Incr. MFO activity (3 mo.)
300	NC	--------	NC	--------
500	--------	Increases: MFO activity (3, 6 mo.), microsomal prot. content (6 mo.), abs. + rel. liver wts. (3 mo.). nonneoplastic liver effects (see table).	--------	Increases: SGPT (3 mo.), MFO activity (3,6,12 mo.), hepatic microsomal prot. content (6 mo.), abs. + rel. liver wts. (3 mo.) Nonneoplastic liver effects (see table).

-------- - Dose not tested in this study
NC - no change

Comparative Toxicity

MICE (CONTINUED)

	MALE		FEMALE	
PPM	90-DAY	2-YEAR	90-DAY	2-YEAR
1000	Increases: abs. + rel. liver wts., MFO activity. Decreases: cholesterol, body wt. gain. Liver effects (see table). Cytoplasmic eosinophilia and/or hypertrophy of zona fasculata cells (adrenals)	--------	Increases: rel. liver wts.	--------
3000	Same as above plus Increases: SGPT. Pigment in macrophages + lymphoid necrosis (2 males) in spleen.	--------	Same as above plus Increases: abs. liver wts MFO activity. Decreases: glucose, cholesterol, body weight gain. Same changes in adrenals as males. Pigment in macrophages in spleen. Liver effects (see table)	--------
10000	Same as above except no lymphoid necrosis of spleen. Increases: MCHC, seg. platelets, SGOT, ALK, GGT, BUN, mononucl. cell infilt. of skin.	--------	Same as above plus Increases: MCHC, platelets, SGOT, SGPT, ALK, GGT, BUN, myeloid:erythroid ratio (granulocytes in marrow), mononucl. cell infilt. of skin.	--------

Comparative Toxicity

MICE (CONTINUED)

	MALE		FEMALE	
PPM	90-DAY	2-YEAR	90-DAY	2-YEAR
10000 cont.	Decreases: bw's, abs. kidney wts, fd. consump. (wk.1), hematocrit, MCV, MCH WBC, lymphocytes, glucose. Pigment in macrophages in kidney.	--------	Decreases: food consump., hematocrit, MCV, MCH, hemoglobin. Lymphoid necrosis in spleen (1), pigment in macrophages in kidney, immaturity of uterus + absence of corpora lutea, increased lymphoid necrosis in thymus.	--------

TABLE II
Mouse 90-Day Feeding Study
Incidence of Nonneoplastic Microscopic Findings in the Liver

Dose Level (ppm)	0		3		10		30		100		300		1000		3000		10,000	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
# Livers Examined	10	10	0	0	0	0	0	0	10	10	10	10	10	10	10	10	10	10
Centrilobular Hepatocytic Hypertrophy	6	0	0	0	0	0	0	0	4	0	5	0	10	0	10	10	0	0*
Microgranuloma (5) Lymphoreticular Cell Infilt.	10	9	0	0	0	0	0	0	2	7	5	9	5	5	5	5	0	1
Swollen Vacuolated Centrilobular Hepatocytes	0	0	0	0	0	0	0	0	0	0	0	0	3	0	10	5	10	10
Single Large Vacuoles, Hepatocytes	0	0	0	0	0	0	0	0	0	0	0	0	3	0	6	8	5	4
Individual Hepatocytic Necrosis, Centrilobular	0	0	0	0	0	0	0	0	0	0	0	0	3	0	8	2	0	0
Coagulative Necrosis	1	2	0	0	0	0	0	0	0	2	0	3	2	1	6	3	1	1
Pigment, Kupffer's Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	10	9
Foci of Granulopoeisis	1	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0	0	0
Congestion	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Periportal Granulomatous Inflammation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1
Centrilobular/Midzonal Hepatocytic Hypertrophy	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	10*
Necrotic Hepatitis, Centrilobular	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	2	10	10
Bile Ductule Proliferation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	7

* High dose animals had both centrilobular and midzonal hepatocytic hypertrophy

TABLE III

Mean Body Weights in a 3-Month Dietary Study
with RH-3866 in Mice (Goldman, et al., 1983)

	Mean Body Weights (g)							
	Control		1000 ppm		3000 ppm		10,000 ppm	
Week	Male	Female	Male	Female	Male	Female	Male	Female
0	33.4	24.9	33.8	24.3	33.2	25.4	33.4	24.9
1	34.6	25.4	35.4 (102%)[a]	25.2 (99%)	33.3 (96.2%)	26.0 (102%)	27.4* (79%)	21.3* (84%)
4	36.4	26.8	37.2 (102%)	26.7 (100%)	34.5 (95%)	26.1 (97%)	27.3* (75%)	23.7* (38%)
13	39.0	28.6	37.3 (96%)	28.6 (100%)	36.4 (93%)	27.8 (97%)	31.6* (81%)	26.2* (92%)

* Statistically significant from control group ($p<0.05$)

Weekly checks indicated the following:
1000 ppm: Males: Mean weekly body weights stayed above 95% of controls
Females: Mean weekly body weights stayed above 97% of controls
3000 ppm: Males: Mean weekly body weights stayed above 91% of controls
(Most values statistically significant between weeks 14-84)
Females: Mean weekly body weights stayed above 97% of controls

Body Weight Gain in a 3-Month Dietary Study[b]
With RH-3866 in Mice (Goldman, et al., 1983)

	Body Weight Gain From Day Zero (g)							
	Control		1000 ppm		3000 ppm		10,000 ppm	
Week	Male	Female	Male	Female	Male	Female	Male	Female
1	1.2	0.5	1.6 (133%)[a]	0.9 (180%)	0.1 (8.3%)	0.5 (120%)	-6.0 -(500%)	-3.6 -(720%)
4	3.0	1.9	3.4 (113%)	2.4 (126.3%)	1.3 (43%)	0.7 (36%)	-6.1 -(203%)	-1.2 -(63%)
13	5.6	3.7	3.5 (63%)	4.3 (116%)	3.2 (57%)	2.4 (65%)	-1.3 -(32%)	1.3 (35%)

a The values in parentheses is the % of the control group value.
b Taken from a presentation delivered by Rohm and Haas

22

TABLE IV

Mean Body Weight Gain (g) and Mean Body Weights (Percent Control Value)
in a 3-Month Dietary Study With RH-3866 in Male Mice
1000 ppm Versus Controls Starting at Day 28

Day	0	28	35	42	49	56	63	70	77	84	91
Body Weight Gain (g)											
Controls	-	3.0	3.4	4.2	3.7	4.3	5.0	5.6	5.3	5.5	5.6
1000 ppm	-	3.4	3.7	3.9	3.7	4.4	4.0	4.0	3.8	3.3	3.5
	-	(113)[a]	(108)	(93)	(100)	(102)	(80)	(71)	(72)	(60)	(63)
Body Weight											
% Control Value	101	102	102	100	101	101	98	97	97	95	96

a The value in parentheses is the % of the control group value

Weekly Body Weight Gain (g) from Day 56 to Day 91
Individual Animal Data for Males at 1000 ppm

Animal Number	18400	18423	18434	18442	18448	18490	18493	18497	18509
Day 56 - Day 63	-0.5	-0.2	-0.6	-0.4	-1.5	+0.2	-0.2	+0.1	0.0
Day 63 - Day 70	+0.3	+0.7	-2.2	+1.6	-1.1	+0.2	+0.2	+0.3	-0.2
Day 70 - Day 77	+0.4	-0.1	-0.4	-2.0	-0.7	-0.2	0.0	+1.0	+0.4
Day 77 - Day 84	+0.3	-0.7	-1.5	-1.7	-1.0	+0.1	+0.5	-0.6	-0.3
Day 84 - Day 91	+0.4	+1.4	-0.9	-0.9	+0.3	+0.8	+0.5	-0.1	-0.1

Summary of Nonneoplastic Liver Effects Observed in Mice After Chronic Exposure to RH-3866

Observed Effect	Dose Level							
	Males				Females			
	0 ppm	20 ppm	100 ppm	500 ppm	0 ppm	20 ppm	100 ppm	500 ppm
Hepatocellular Centrilobular Hypertrophy								
3 months	1/10	1/10	1/10	9/10	0/10	0/10	0/10	0/10
6 months	2/10	2/10	1/10	9/10	0/10	0/10	0/10	0/10
12 months	5/20	6/20	5/20	16/20	1/20	0/20	1/20	2/20
12-24 months	8/66	6/63	5/65	11/62	0/64	0/66	0/66	0/67
Sentinel (12 mo.)	1/5	-	-	-	0/5	-	-	-
Kupffer Cell Pigmentation								
3 months	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
6 months	0/10	0/10	0/10	5/10	0/10	0/10	0/10	0/10
12 months	4/20	1/20	4/20	12/20	4/20	2/20	1/20	4/20
12-24 months	0/66	0/63	0/65	0/62	0/64	0/66	0/66	0/67
Sentinel (12 mo.)	2/5	-	-	-	0/5	-	-	-
Periportal Punctate Vacuolation								
3 months	0/10	0/10	0/10	2/10	0/10	0/10	0/10	1/10
6 months	0/10	0/10	0/10	3/10	0/10	0/10	1/10	2/10
12 months (multifocal)	0/20	0/20	0/20	4/20	0/20	1/20	1/20	3/20
12-24 months	0/66	0/63	0/65	0/62	0/64	0/66	0/66	0/67
Sentinel (multifocal)	0/5	-	-	-	1/5	-	-	-
Individual Hepatocellular Necrosis								
3 months	1/10	1/10	3/10	3/10	5/10	3/10	1/10	3/10
6 months	1/10	1/10	3/10	3/10	0/10	0/10	0/10	0/10
12 months (multiple)	2/20	1/20	1/20	6/20	0/20	0/20	1/20	2/20
12-24 months	0/66	0/63	0/65	0/62	0/64	0/66	0/66	0/67
Sentinel (12 mo.)	2/5	-	-	-	0/5	-	-	-

Observed Effect	Dose Level							
	Males				Females			
	0 ppm	20 ppm	100 ppm	500 ppm	0 ppm	20 ppm	100 ppm	500 ppm
Focal Hepatocellular Alterations								
3-12 months	0/40	0/40	0/40	0/40	0/40	0/40	0/40	0/40
Sentinel (12 mo.)	0/5	-	-	-	0/5	-	-	-
Terminal Sacrifice								
focus/foci, basophilic	2/66	3/63	1/65	4/62	0/64	0/66	1/66	2/67
focus/foci, clear-cell	0/66	0/63	0/65	2/62	0/64	0/66	0/66	0/67
focus/foci, eosinophilic	2/66	1/63	4/65	5/62	2/64	2/66	1/66	4/67
focus/foci, vacuolated cell	0/66	0/63	1/65	0/62	0/64	0/66	0/66	0/67
Total incidence	4/66	4/63	5/6/65*	10/11/62*	2/64	2/66	2/66	6/67
Multifocal Hepatocellular Vacuolation								
3-12 months	0/40	0/40	0/40	0/40	0/40	0/40	0/40	0/40
Sentinel (12 mo.)	0/5	-	-	-	0/5	-	-	-
Terminal Sacrifice								
centrilobular	1/66	1/63	0/65	1/62	0/64	1/66	0/66	0/67
diffuse	0/66	0/63	0/65	0/62	0/64	0/66	0/66	1/67
multifocal	1/66	0/63	2/65	7/62	3/64	2/66	0/66	7/67

* / - number of mice with hepatocellular alteration/actual incidence of hepatocellular alteration. In 4 instances, a mouse had more than 1 type of hepatocellular alteration (or neoplasia, which were not included in this table).

TABLE VI

Mean Body Weights in the Chronic Feeding/Oncogenicity Study With RH-3866 In Mice

	Mean Body Weights (g)			
	Control		500 ppm	
Week	Male	Female	Male	Female
0	28.9	23.0	28.9 (100)[a]	23.0 (100)
13	37.3	30.8	37.0 (99.2)	30.1 (98)
24	39.0	32.8	38.6 (99)	31.4 (96)
38	39.7	33.5	39.4 (99)	33.6 (100)
52	40.4	34.8	39.9 (99)	33.7 (97)

a The value in parentheses is the % of the control group value.

Mean Body Weight Gains in the Chronic Feeding/Oncogenicity Study With RH-3866 in Mice

	Mean Body Weight Gains (g)			
	Control		500 ppm	
Week	Male	Female	Male	Female
13	8.4	7.8	8.1 (96%)[a]	7.1 (91%)
24	10.1	9.8	9.7 (96%)	8.4 (86%)
38	10.8	10.5	10.5 (97%)	10.6 (100%)
52	11.5	11.8	11.0 (96%)	10.7 (90.6%)

a The value in parentheses is the % of the control group value.

B. Rat

[See Tables 7-11 for comparative summary of toxicity and Appendix I for DERs of these studies].

1. 90-Day Feeding Study - rat (Core Grade Minimum)

Strain: COBS-CD(SD) BR
Age: 25-28 days plus 4 weeks more for quarantine
Weight: Not given
Source: Charles River Breeding Labs, Kingston, N.Y.
Testing Facility: Rohm & Haas Toxicology Dept.
Spring House, PA
Total rats tested in study: 180

Groups of 10 rats/sex were fed control diets and test diets containing the following levels of RH-3866: 5/7/10, 15/21/30, 50/70/100, 150/210/300, 500/700/1000, 1500/2100/3000, 5000/7000/10000, and 15000/21000/30000 ppm. The first dose at each level was fed for 2 weeks, the second dose was fed for 2 weeks and the third dose was given for the remainder of the test period.

The NOEL for liver mixed function oxidase is 100 ppm in males and 300 ppm in females. The LEL's are 300 ppm and 1000 ppm, respectiviely. The NOEL for other liver effects is 1000 ppm for both sexes. At this dose level, only accentuated liver architecture was grossly observed in males and increases in relative liver weights were observed in females. The LOEL is 3000 ppm based upon decreases in body weight gain in both sexes, and effects in the liver, kidneys, adrenals and thyroid (see Tables 7-9 for details).

All animals at the 30000 ppm dose level died during the study. There were no treatment-related effects on survival at any of the other dose levels. Clinical signs of toxicity were observed in the animals which died, but no signs of clinical toxicity were observed at any of the other dose levels. Hematological effects and decreases in food consumption were observed at 10000 ppm in both sexes, increases in cholesterol and globulin and various organ weights were observed at 3000 ppm and an increase in BUN was observed at day 32 but not at day 91 when both sexes were combined at 3000 ppm. Changes in other clinical chemistry parameters were noted at 10000 ppm. See Table 7 for details.

2. 2-Year Chronic Feeding/Oncogenicity Study - Rat
(Core Grades: Chronic - Guideline; Oncogenicity - Reserved)

Strain: Sprague-Dawley
Age: 8-11 weeks after acclimization period
Weight: 130-140 gms, 1 week prior to initiation of study
Source: Charles River Breeding Labs, Wilmington, MA
Testing Facility: Tegeris Laboratories, Laurel, MD
Total mice tested in study: 52 males/group (4 groups), 60 females/group (4 groups); 10/sex/group for interim sacrifices at 3 and 6 months, 20/sex/group for interim sacrifice at 12 months, and 18 males/group and 10 females/group for interim sacrifice at 17 months. Thirty animals of each sex were used in the sentinel program.

Rats were treated up to 24 months with the following dose levels: 0, 25/35/50, 100/140/200, and 400/560/800 ppm. The first dose at each level was fed for 2 weeks, the second dose at each level was fed for 2 weeks and the third dose at each level was fed for the remainder of the test period. The overall mean daily consumption was 0, 2.49, 9.84 and 39.21 mg/kg/day for males and 0, 3.23, 12.86 and 52.34 mg/kg/day for females.

No oncogenic effects were observed.

Maximum Tolerated Dose (MTD) - This issue is reserved pending the decision made by the review committee following the review of this study and the 90-day study.

The NOEL for the study is 2.49 mg/kg/day (50 ppm) in males based upon testicular atrophy. In females, the NOEL for increased MFO activity is 3.23 mg/kg/day (50 ppm). The NOEL for other effects in females is 52.34 mg/kg/day (800 ppm). The only effect seen in females at this dose level was increased liver weights at 3 and 6 months. The LOEL for testicular atrophy is 9.84 mg/kg/day (200 ppm) and the LOEL for MFO activity in females is 12.86 mg/kg/day. See Tables 7, 10 and 11 for details.

There were no treatment-related effects on survival or clinical signs of toxicity. In males, there appeared to be a slight effect of the treatment on bodyweights between 6 and 18 months. Although the body weights were statistically significantly less than controls, they were still within 95-97% of the control values. In females, there may have been a slight effect on bodyweights in the second year of the study at the high dose. The bodyweights were generally lower than controls during weeks 54-96 and the differences were statistically significant at weeks 66-72, 76-84 and 91. During weeks 76-84, the body weights were generally between 88-91% of the control values. A slight decrease in food consumption was observed in high dose males. Other than

corresponding decreases in testicular weights and increased MFO activity in males, no other changes were observed in clinical chemistries, hematology, urinalysis or in organ weights.

TABLE VII

Comparative Toxicity

RATS

	MALE		FEMALE	
PPM	90-DAY	2-YEAR	90-DAY	2-YEAR
5/7/10*	NC	--------	NC	--------
15/21/30	NC	--------	NC	--------
25/35/50	--------	NC	--------	NC
50/70/100	NC	--------	NC	--------
100/140/200	-------	Decreases: testicular wts, testes-bw ratios. Testicular atrophy (see table)	--------	Increases: MFO activity (3 mo.)
150/210/300	Increases: MFO activity	--------	NC	--------
400/560/800	--------	Same as above plus Increases: MFO activity (3,6 mo.). Decreases: bw (sl.); fd. consump. (sl.)	--------	Same as above plus Increases: liver-bw ratio (3 mo.), liver wts. (6 mo.) Decreases: bodyweight (sl.)

-------- = dose not tested in this study NC = No change

*In both the 90-day study and the 2-year study, the first dose was fed for 2 weeks, the second dose for 2 weeks, and the third dose for the remainder of the study.

Comparative Toxicity

RATS (CONTINUED)

	MALE		FEMALE	
PPM	90-DAY	2-YEAR	90-DAY	2-YEAR
500/700/1000 Same as above plus accentuated liver architecture (gross observation).	--------		Increases: MFO activity, rel. liver wts.	--------
1500/2100/3000 Same as above plus Increases: cholesterol, globulin, rel. kidney wts., rel. + abs. liver wts., rel. thyroid wts., BUN (day 32, comb. sexes). Decreases: bw gain, abs. adrenal wts.. Liver effects (see table), pigmentation of convolut. tubul. epith. of kidney, vacuolation of adrenal cortices, incr. in small follicles of thyroid.	--------		Same as above plus Increases: rel. heart + kidney wts, abs. liver wts., BUN (day 32, comb. sexes). Decreases: bw gain. Liver effects (see table), vacuolation of adrenal cortices.	--------
5000/7000/10000 Same as above plus Increases: platelets (sl.), SGPT, AP (comb. sexes), BUN, Ca, GGT, total prot., rel. gonad wts. Decreases: bw, fd. consump., MCV (sl.) hemoglobin. Increase in frequency of chronic alveolitis.	--------		Same as above plus Increases: platelets (sl) cholesterol, AP (comb. sexes), BUN, GGT, total protein, globulin, rel. gonad wts, rel. thyroid wts, rel. spleen wts. Decreases: bw, fd. consumption, MCV, hemoglob., A/G ratio.	--------

Comparative Toxicity

RATS (CONTINUED)

PPM	MALE 90-DAY	MALE 2-YEAR	FEMALE 90-DAY	FEMALE 2-YEAR
5000/7000/10000 (cont.)		--------	Accentuated liver architecture, increase in frequency of chronic alveolitis.	--------
15000/21000/30000 100% mortality		--------	100% mortality	--------

TABLE 8. Summary of Incidence of Liver Lesions in Male and Female Rats Fed RH 3866 for 90 Days

Dose: (ppm)	Control		300		1000		3000		10,000	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals examined:	10	10	10	0	10	10	10	10	10	10
Centrolobular hypertrophy with increased eosinophilia	0	0	0	-	0	0	10	7	10	10
Vacuolated swollen hepatocytes	0	0	0	-	0	0	0	0	9	0
Hepatocellular necrosis	0	0	0	-	0	0	1	3	1	1
Fatty metamorphosis	0	0	0	-	2	1	1	0	0	0
Necrosis, coagulation, zones	1	0	0	-	0	0	0	0	2	0

table IX

Mean Body Weights in a 3-Month Dietary Study
with RH-3866 in Rats (O'Hara and DiDonato)

	Mean Body Weights (g)							
	Control		1000 ppm		3000 ppm		10,000 ppm	
Week	Male	Female	Male	Female	Male	Female	Male	Female
0	284	184	295	179	292	181	298	181
1	320	200	333 (104%)[a]	196 (98%)	324 (101%)	197 (99%)	294* (92%)	193* (97%)
4	407	238	408 (100%)	230 (97%)	393 (97%)	229 (96%)	335* (82%)	223* (94%)
13	517	285	508 (98%)	278 (98%)	476 (92%)	269 (94%)	365* (71%)	244* (86%)

* Statistically significant from control group ($p<0.05$).

Weekly checks indicated the following:
1000 ppm: Males: Mean bodyweights stayed above 96 of controls
Females: Mean bodyweights stayed above 93% of controls
3000 ppm: Males: Mean bodyweights stayed above 91% of controls.
(Statistically significant from controls at weeks 42-84).
Females: Mean bodyweights stayed above 93% of controls

Body Weight Gain in a 3-Month Dietary Study[b]
With RH-3866 in Rats (O'Hara and DiDonato, 1984)

	Body Weight Gain From Day Zero (g)							
	Control		1000 ppm		3000 ppm		10,000 ppm	
Week	Male	Female	Male	Female	Male	Female	Male	Female
1	36	16	38 (105%)[a]	17 (106%)	32 (89%)	16 (100%)	-4 -(11%)	12 (75%)
4	113	54	113 (100%)	51 (94%)	101 (89%)	48 (88%)	37 (33%)	42 (78%)
13	223	101	213 (96%)	99 (98%)	184 (83%)	88 (87%)	67 (30%)	63 (62%)

a The values in parentheses is the % of the control group value.
b Taken from a presentation delivered by Rohm and Haas

34

TABLE 10
RAT CHRONIC FEEDING/ONCOGENICITY STUDY
INCIDENCE OF UNILATERAL AND BILATERAL TESTICULAR ATROPHY

	Control	Low	Mid	High
		12-Month Sacrifice		
Bilateral	0/20	0/19	1/20	3/20
Unilateral	0/20	1/19	0/20	0/20
		17-Month Sacrifice		
Bilateral	2/18	2/18	0/18	4/18
Unilateral	2/18	2/18	0/18	1/18
		Terminal Sacrifice		
Bilateral	2/17	1/19	5/20	12/22
Unilateral	2/17	3/19	6/20	2/22
		Animals That Died or Were Sacrificed Moribund		
Bilateral	1/35	4/35	10/32	12/30
Unilateral	6/35	4/35	5/32	5/30
		Total Incidence of Testicular Atrophy Across All Groups*		
Bilateral	5/110	7/110	16/110	31/110
Unilateral	10/110	10/110	11/110	8/110

* Including 3 and 6 month sacrifices (10 animals apiece, except low dose at 3 months had only 9 animals).

TABLE XI

Mean Body Weights in the Chronic Feeding/Oncogenicity Study With RH-3866 In Rats

	Mean Body Weights (g)			
	Control		800 ppm	
Week	Male	Female	Male	Female
0	190.2	163.9	188.3 (99)[a]	163.1 (100)
13	526.3	297.6	515.5 (98)	297.2 (100)
26	637.0	348.8	611.1[b] (96)	349.5 (100)
38	710.2	390.0	674.1[b] (95)	385.4 (99)
52	778.3	443.6	746.0 (96)	439.2 (99)

a The value in parentheses is the % of the control group value.
b Statistically significant from controls ($p<0.05$)

Mean Body Weight Gains in the Chronic Feeding/Oncogenicity Study With RH-3866 in Rats

	Mean Body Weight Gains (g)			
	Control		800 ppm	
Week	Male	Female	Male	Female
13	336.0	133.7	327.2 (97%)[a]	134.1 (100%)
26	446.7	184.9	422.8 (95%)	186.4 (100%)
38	520.0	226.1	485.8 (93%)	222.3 (98%)
52	588.0	279.7	557.7 (95%)	276.1 (99%)

a The value in parenthesis is the % of the control group value.

C. Discussion of Rodent Studies and Summary of Registrant's Rationale for Selection of Dose Levels for Oncogenicity Studies

In the 2-year mouse feeding study (doses 20, 100 and 500 ppm), there was no indication of oncogenic effects in either sex. The changes observed were increases in liver MFO activity (starting at 100 ppm for females and at 500 ppm for males), and increases in liver weights and other liver effects at 500 ppm in both sexes. The liver effects included hepatocellular hypertrophy, Kupffer cell pigmentation, periportal punctate vacuolation, individual hepatocellular necrosis, focal hepatocellular alterations and multifocal hepatocellular alterations. The individual hepatocellular necrosis did not appear at terminal sacrifice. It was slightly increased in both incidence (6/20) and severity in male mice fed 500 ppm for 12 months when compared to control males (2/20). The necrosis was characterized by single scattered hepatocytes with pyknotic, karyorrhectic or karyolytic nuclei. A few inflammatory cells, predominantly neutrophils, were occasionally present around or within the necrotic hepatocytes which were predominantly located in centrilobular areas.

Dose selection of the chronic study was based on a 90-day feeding study in the mouse (doses 3, 10, 30, 100, 300, 1000, 3000, and 10000 ppm). In this study, no changes were observed in dose levels up to and including 300 ppm. At 1000 ppm, males had a significant decrease in body weight gain, increases in liver weights and MFO activity, hepatocytic hypertrophy, swollen-vacuolated centrilobular hepatocytes and individual hepatocytic necrosis, centrilobular (3/10), as well as cytoplasmic eosinophilia and/or hypertrophy of the zona fasculata cells of the adrenals. Females only had increases in relative liver weights. At 3000 ppm, females began to exhibit the same effects as males, including the decrease in bodyweight gain. The Registrant chose the top dose of 500 ppm for the chronic study because they believed that 3000 ppm definitely exceeded the MTD and that 1000 ppm probably exceeded the MTD as indicated by the 37% decrease in body weight gain in males and the liver toxicity in both sexes. The Agency disagrees that significant liver toxicity was seen at 1000 ppm for the females. One-half of the 1000 ppm was therefore, chosen by the Registrant to be the top dose in the chronic mouse study.

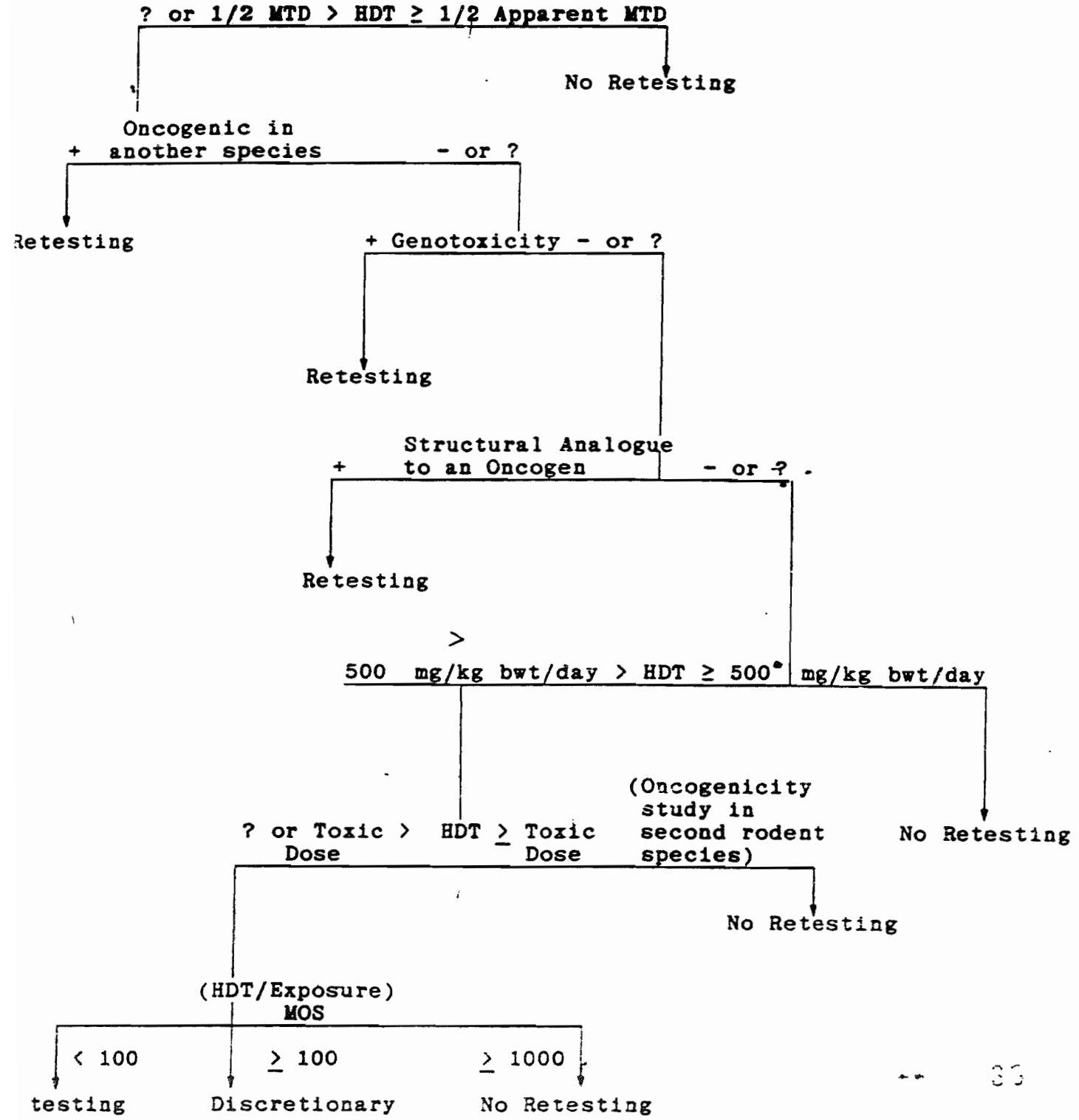
In the 2-year rat feeding study (doses 50, 200 and 800 ppm), there was no indication of oncogenic effects in either sex. The changes observed were testicular atrophy in males at levels of 200 ppm and above and increased MFO activity at levels of 200 ppm and above in females and 800 ppm in males. Increased liver weights were seen in females at 800 ppm at 3 and 6 months and decreased testicular weights were observed in males at 200 ppm and above. There appeared to be a slight effect of the test chemical on the body weights in both sexes at the highest dose level.

Dose selection for the rat chronic feeding study was based on a 90-day feeding study in the rat (doses 10, 30, 100, 300, 1000, 3000, 10000 and 30000 ppm). In this study, no changes were observed in either sex at doses up to and including 100 ppm. At 300 ppm and above, increases in MFO activity in males was observed. At 1000 ppm, accentuated liver architecture was grossly observed in males and females exhibited an increase in relative liver weights and well as increases in MFO activity. The report pathologist stated that the NOEL for liver effects was 1000 ppm. At 3000 ppm and above, animals in both sexes exhibited decreases in body weight gain and effects in the liver, kidneys, adrenals and thyroid. At 10000 ppm, additional effects were observed in the hematology and clinical chemistry studies. The Registrant estimated the MTD to be 1000 ppm, where moderate toxicity was seen (the Registrant had made an error in calculations here and had originally seen a 20% decrease in body weight gain in females). On the basis of the estimated 1000 ppm MTD, the Registrant selected 800 ppm to be the top dose in the chronic study.

In a position paper on the evaluation of the adequacy of the highest dose tested in the two oncogenicity studies, the Registrant presented a tier scheme for requiring new oncogenicity testing for completed studies without an MTD. The tier scheme was endorsed by the SAP on May 22, 1986. They state that on the basis of the tier scheme, the studies need not be repeated. The tier scheme is presented in Figure 3. The two following pages represent the Registrant's position on the basis of the tier scheme as to why the studies should not be repeated.

FIGURE III

Tier Scheme for Requiring New Oncogenicity Testing for Completed Studies without a Maximum Tolerated Dose (MTD)



III. Evaluation of the adequacy of the HDT in accordance with the draft 1986 EPA Position Paper on MTD - Tier scheme to determine if a study completed or in progress need to be repeated

A. Mouse Oncogenicity Study with RH-3866:

Level 1: Nearness to the Apparent MTD

- 500 ppm $\geq$ 1/2 MTD
- Thus the study need not to be repeated

Level 2: Demonstrated Oncogenicity

- No study with RH-3866 has been demonstrated oncogenicity
- Thus a "-" is applied

Level 3: Genotoxicity

- RH-3866 was not genotoxic in a battery of assays
- Thus a "-" is applied

Level 4: Oncogenicity of Structural Analogs

- Triazole/imidazole fungicides are generally not oncogenic
- Thus a "-" is applied

Level 5: Absolute Value of Highest Dose Tested

- 500 ppm or 75 mg/kg/day < 500 mg/kg/day

Level 6: Highest Dose Tested Relative to Dose Tested in Second Species of an Oncogenicity study with MTD

- Both rat and mouse studies are under review by EPA
- Thus a "+/-" is applied

Level 7: Margin of Safety Calculated for Highest Dose Tested vs. Human Exposure

- Human Dietary Exposure Estimate = 0.0012 mg/kg/day based on Tolerances
- Margin of Safety for HDT = 75 mg/kg/day $\div$ 0.0012 mg/kg/day
  = 62,000
  >> 1000
- Thus the study need not to be repeated

III. Evaluation of the adequacy of the HDT in accordance with the draft 1986 EPA Position Paper on MTD - Tier scheme to determine if a study completed or in progress need to be repeated

A. Rat Oncogenicity Study with RH-3866:

Level 1: Nearness to the Apparent MTD

- 800 ppm > 1/2 MTD
- Thus the study need not to be repeated

Level 2: Demonstrated Oncogenicity

- No study with RH-3866 has been demonstrated oncogenicity
- Thus a "-" is applied

Level 3: Genotoxicity

- RH-3866 was not genotoxic in a battery of assays
- Thus a "-" is applied

Level 4: Oncogenicity of Structural Analogs

- Triazole/imidazole fungicides are generally not oncogenic
- Thus a "-" is applied

Level 5: Absolute Value of Highest Dose Tested

- 800 ppm or 40 mg/kg/day < 500 mg/kg/day

Level 6: Highest Dose Tested Relative to Dose Tested in Second Species of an Oncogenicity study with MTD

- Both rat and mouse studies are under review by EPA
- Thus a "+/-" is applied

Level 7: Margin of Safety Calculated for Highest Dose Tested vs. Human Exposure

- Human Dietary Exposure Estimate = 0.0012 mg/kg/day based on Tolerances
- Margin of Safety for HDT = 40 mg/kg/day ÷ 0.0012 mg/kg/day
  = 33,333
  >> 1000
- Thus the study need not to be repeated

V. Summary

RH-3866 did not exhibit any oncogenic effects in either a chronic feeding study in the rat or in the mouse at the dose levels tested. The issue for the review committee is:

o Do the rat and mouse oncogenicity studies approach the Maximum Tolerated Dose for males and/or females?

Possible choices

MTD not approached

MTD is approached only in one sex

MTD is approached in both sexes

MTD not approached but study is acceptable on the basis of the tier scheme presented

APPENDICES

A. Data Evaluation Reports (DERs)

008060

Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

Budd 1/11/88

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Feeding - mouse TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266079

TEST MATERIAL: RH-3866

SYNONYMS: Rally, Systhane, Myclobutanil

REPORT NUMBER: 83R-136

SPONSOR: Rohm & Haas Co., Philadelphia, PA

TESTING FACILITY: Rohm & Haas Toxicology Dept., Spring House, PA

TITLE OF REPORT: RH-3866: A Three-Month Dietary Toxicity Study in Mice

AUTHOR(S): P.R. Goldman, J.C. Harris, K.R. Lampe

REPORT ISSUED: October 8, 1986

IDENTIFYING VOLUME: Vol. 5 of 47

CONCLUSION: The NOEL for this study is 300 ppm based upon hepatocytic hypertrophy and other liver effects. The LOEL is 1000 ppm. The dose levels tested included levels of 3-10,000 ppm in the diet.

Classification: Guideline

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile
Description: brown-colored solid
Batch #(s), Other #(s): Sample No. 83-076, Lot No. LSPL0016/E
Purity: 81.1%
Source: Rohm & Haas

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Crl:CD®-1(ICR)BR mice, male and female
Age: 8 weeks
Weight(s): 32-34 g (males), 24-26 g (females)
Source(s): Charles River Breeding Labs, Stone Ridge, N.Y.

45

45

3. Procedure:

a. Dietary Preparation (if applicable): A jar of the technical sample is heated each week until liquid (50-60°C). The appropriate amount was weighed, dissolved in 50 ml acetone and mixed with feed in a hood to evaporate the acetone.

Frequency of preparation: weekly

Storage conditions: room temperature

Stability Analyses: Selected samples from the weekly preparations at each dose level were taken for stability analysis.

Homogeneity Analyses: Samples from the top, middle and bottom of each dietary concentration were collected the first time the diets were prepared and submitted for analysis of homogeneity of mixing.

Concentration Analyses: Conducted from the stability analysis studies.

b. Animal Assignment and Dose Levels:

Test Group	Dose Administered (ppm)	Main Study 13 weeks male	female
Contr.	0	10	10
1	3	10	10
2	10	10	10
3	30	10	10
4	100	10	10
5	300	10	10
6	1000	10	10
7	3000	10	10
8	10000	10	10

c. Clinical Observations and Mortality: Observed daily for signs of toxicity. Physical examinations conducted weekly.

d. Body Weight Determinations: weekly

e. Food and/or Water Consumption: weekly

f. Ophthalmological Examinations (if applicable): Not done

g. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals):
At the end of the dosing period (13 weeks)

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	x	Mean corpuscular HGB (MCH)
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB conc.(MCHC)
x	Leukocyte count (WBC)*	x	Mean corpuscular volume (MCV)
x	Erythrocyte count (RBC)*	x	Red cell morphology*
x	Platelet count*		
x	Total plasma protein (TP)		* Only determined for mice in the control group and the 2 highest dose groups (3000 and 10,000 ppm)
x	Leukocyte differential count*		

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X	Electrolytes:	X	Other:
x	Calcium*	x	Albumin*
	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorus*	x	Cholesterol*
	Potassium*	x	Globulins
	Sodium*	x	Glucose*
	Enzymes:	x	Total bilirubin*
x	Alkaline phosphatase	x	Total protein*
	Cholinesterase		Triglycerides
	Creatinine phosphokinase*	x	A/G ratio
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
x	Gamma glutamyl transferase (GGT)		

3) Hepatic Mixed Function Oxidase Assays:

Liver sections were taken from 4 animals/sex/group in each of the six highest dose groups (30 to 10,000 ppm). The liver sections were analyzed for hepatic mixed function oxidase activity through the use of benzphetamine and aminopyrine N-demethylation assays.

4) Urinalysis: Not conducted

h. Gross Necropsy: After the 13 week treatment period, all surviving mice were necropsied and all organs, tissues and body cavities were examined and gross abnormalities were recorded.

i. Histopathology: The tissues marked below were saved from all animals that were necropsied. Microscopic examinations were conducted on all tissues saved from all animals in the control and the two highest dose groups (3000 and 10000 ppm). Target tissues were examined in the lower dose groups until a NOEL was reached.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

X	Digestive system	X	Cardiovasc./Hemat.	X	Neurologic
	Tongue		Aorta*	xx	Brain*
x	Salivary glands*	xx	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	xx	Spleen*	x	Eyes (optic n.)*
x	Jejunum*	x	Thymus*		Glandular
x	Ileum*		Urogenital	xx	Adrenals*
x	Cecum*	xx	Kidneys*		Lacrimal gland
x	Colon*	x	Urinary bladder*	x	Mammary gland*
x	Rectum*	xx	Testes*	xx	Parathyroids*
xx	Liver*		Epididymides	xx	Thyroids*
x	Gall bladder*	x	Prostate		Other
x	Pancreas*	x	Seminal vesicle	x	Bone*
	Respiratory	xx	Ovaries	x	Skeletal muscle*
x	Trachea*	x	Uterus*	x	Skin
x	Lung*			x	All gross lesions and masses

j. Statistical Analyses: Residual plots of the numerical parameters were examined for normality and homogeneity of variance across treatment groups. Analysis of variance was used for the same parameters; some group means were compared using Duncan's Multiple Range Test and other groups were compared using the Least Square Means Test.

B. RESULTS:

1. Dietary Preparation: Samples were taken for analysis from weeks 1 (including samples obtained for homogeneity testing), 2, 3, 4, 8, 12 and 13. No measurable residue of technical RH-3866 was found in most of the control samples; residues up to 9 ppm were found in 3 control samples (considered to be sample contamination). Recovery for 1-day aged samples averaged 94 +/15%. No explanation was given as to why the sample was allowed to age only 1 day. Average dose levels ranged from 90-119% of the theoretical values, with an overall average of 106%. The individual data indicate that there may have been some difficulties in mixing the test chemical into the diet. For example, at the theoretical dose level of

1000 ppm, the measured levels of chemical in the diet were 1450, 1600 and 690 ppm for samples taken from the bottom, middle and top of the mixer, respectively.

2. Clinical Observations and Mortality: One male mouse fed 1000 ppm RH-3866 died during the course of the study. The death was not considered to be treatment-related. The gross pathology report on this animal included a mottled liver, redness of the glandular stomach and duodenum, and red fluid in the abdominal cavity. The only clinical sign which appeared to be treatment-related was the notation of scant fecal droppings in the 10,000 ppm group throughout the dosing period.

3. Body Weight Determinations: At 10,000 ppm, the body weights of both male and female groups were statistically significantly lower than controls at all times except for females at week 7. Although body weight gains were not analyzed, graphical representation indicates that there was a highly significant decrease in the high dose animals when compared to controls during the first week. Thereafter the body weight gains appear to be similar to controls. At 13 weeks, the differences in body weights were approximately 19 and 8% for males and females respectively when compared to control groups. At 3000 ppm, the male body weights were also statistically significantly lower than controls at most time points. These decreases averaged around 7% lower. None of the animals in any of the other dose groups were affected.

4. Food and/or Water Consumption: The food consumption of male mice at the 10,000 ppm level was reduced during the first week. Food consumption of females at this dose level was reduced throughout the treatment period, and was statistically significantly lower during the first week. Differences at other times were considered to be incidental.

5. Hematology: At 10,000 ppm, a significant decrease in the hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) values were observed in both sexes and an increase in mean corpuscular hemoglobin concentration (MCHC) was observed in both sexes. At this dose level, males also had a significant decrease in WBC and the number of lymphocytes and an increase in the number of segmented platelet values, and females had decreased hemoglobin and increased platelet values. For various reasons, none of the other observed changes were considered to be related to treatment.

6. Clinical Chemistry: At 10,000 ppm, increases in SGOT, SGPT, ALK, SGT and BUN were observed in both sexes. Increases in SGPT were also observed in both sexes at 3000 ppm and in males at 1000 ppm, although not statistically significant in females at 3000 ppm or in males at 1000 ppm. SGOT was increased in males at 3000 ppm, although not significantly so. Glucose levels were significantly reduced in both sexes at 10,000 ppm and in females at 3000 ppm. Cholesterol

values were decreased in both sexes at 10,000 ppm and 3000 ppm and in males at 1000 ppm. For various reasons, none of the other changes observed were considered to be related to treatment.

7. Gross Pathology: At 10,000 ppm and at 3000 ppm enlarged livers with accentuated lobular architecture were observed in both sexes. At 10,000 ppm, 18/20 animals were affected (20/20 with accentuated lobular architecture), and at 3000 ppm, 4/20 animals were affected. Other abnormalities observed were not considered to be treatment-related.

8. Organ Weights: Liver weights (absolute and relative) were increased in both sexes at the 1000, 3000 and 10,000 ppm dose levels. All increases were statistically significant except the absolute liver weights in 1000 ppm female mice. Absolute kidney weights were decreased in male mice at 10,000 ppm. No other changes in organ weights at any dose level were considered to be treatment-related. Some differences were considered to be probably due to decreased terminal body weights in some animals.

9. Hepatic Mixed Function Oxidase Assay: RH-3866 increased MFO activity on a per g liver basis (BP N-demethylation) in females at 300, 3000 and 10,000 ppm but had no effect at 1000 ppm. Therefore, the increase at 300 ppm was not considered to be biologically significant. MFO activity was increased in males at levels of 1000 ppm and greater.

10. Histopathology:

a. Nonneoplastic lesions: Microscopic changes in the liver were seen in males at dose levels of 1000 ppm and above and in females at dose levels of 3000 ppm and above. These observations included centrilobular or centrilobular and midzonal hepatocytic hypertrophy, swollen-vacuolated centrilobular hepatocytes, single large hepatocytic vacuoles, centrilobular individual cell hepatocytic necrosis and centrilobular necrotic hepatitis. Pigment in Kupffer cells was evident in both sexes at 3000 and 10,000 ppm. Bile duct proliferation was seen in both sexes at 10,000 ppm. The NOEL for liver effects. was 300 ppm in males and 1000 ppm in females. The microscopic findings for liver are summarized in table 1.

Other observed changes that were considered to be compound-related were cytoplasmic eosinophilia and/or hypertrophy of the zona fasculata cells of the adrenal glands at 1000 ppm in males and at 3000 ppm and above in both sexes; the presence of pigment in the macrophages in the spleen (3000 ppm and above) and kidney (10,000 ppm); lymphoid necrosis in the spleen (2 males at 3000 ppm, 1 female at 10,000 ppm and 3 of each sex at 30,000 ppm); an increase in the myeloid:erythroid ratio, primarily involving the granulocytes in the bone marrow in some females at 10,000 ppm; immaturity of the uterus and absence of corpora lutea in the ovaries of females at

10,000 ppm and increased mononuclear cell infiltration in the skin in both sexes at 10,000 ppm. All other changes were considered to be spontaneous in nature and unrelated to treatment.

b. Neoplastic lesions: None

11. Quality Assurance Measures: The report and original data were reviewed by the Quality Assurance Unit of Rohm and Haas. To the best of their knowledge, the study did not deviate from the published GLP's. The report was signed.

C. DISCUSSION: The major compound-related effect observed in this study is hepatocytic hypertrophy, along with other liver effects. These effects were evident both on a gross level and on a microscopic level. In addition, they were supported by clinical chemistry evidence and by the organ weight data. There were several questions concerning the design and conduct of the study and there were slight deviations from the EPA Testing Guidelines, but these were not considered to be significant enough to affect the outcome of the study. There appeared to be a possible problem with homogeneity of mixing. This is pointed out in the results section. In addition, the stability analysis study was done for a sample which was only stored for one day. Since the diets were formulated weekly, the sample tested was not stored long enough prior to testing. No explanation was given as to why the test sample was not stored for at least a week. The Guidelines called for an ophthalmological examination. This was not done. The Guidelines also called for microscopic examinations on the lungs, livers and kidneys of all test groups. Only the liver and other target organs were examined microscopically in the other test groups besides the 2 top dose levels. Since all organs were examined in the 2 top test groups and since target organs were examined in the lower dose levels (at least until a NOEL was reached), this deviation from the Guidelines is not considered to be significant. This study is classified as CORE GUIDELINE.

51

Dose Level (ppm)	0		3		10		30		100		300		1000		3000		10,000	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
# Livers Examined	10	10	0	0	0	0	0	0	10	10	10	10	10	10	10	10	10	10
Centrilobular Hepatocytic Hypertrophy	6	0	0	0	0	0	0	0	4	0	5	0	10	0	10	10	0	0*
Microgranuloma (5) Lymphoreticular Cell Infilt.	10	9	0	0	0	0	0	0	2	7	5	9	5	5	5	5	0	1
Swollen-Vacuolated Centrilobular Hepatocytes	0	0	0	0	0	0	0	0	0	0	0	0	3	0	10	5	10	10
Single Large Vacuoles, Hepatocytes	0	0	0	0	0	0	0	0	0	0	0	0	3	0	6	8	5	4
Individual Hepatocytic Necrosis, Centrilobular	0	0	0	0	0	0	0	0	0	0	0	0	3	0	8	2	0	0
Coagulative Necrosis	1	2	0	0	0	0	0	0	0	2	0	3	2	1	6	3	1	1
Pigment, Kupffer's Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	10	9
Foci of Granulopoeisis	1	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0	0	0
Congestion	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Periportal Granulomatous Inflammation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1
Centrilobular/Midzonal Hepatocytic Hypertrophy	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	10*
Necrotic Hepatitis, Centrilobular	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	2	10	10
Bile Ductule Proliferation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	7

* High dose animals had both centrilobular and midzonal hepatocytic hypertrophy

h. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals):
3, 6, 12 and 24 months; 10/sex/group at 3 and 6 months, 15/sex/group at 12 and 24 months.

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	x	Mean corpuscular HGB (MCH)
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB conc.(MCHC)
x	Leukocyte count (WBC)*	x	Mean corpuscular volume (MCV)
x	Erythrocyte count (RBC)*	x	Red cell morphology†
x	Platelet count*		
	Total plasma protein (TP)		
x	Leukocyte differential count*†		†Only on high dose and controls

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X		X	
	Electrolytes:		Other:
x	Calcium*	x	Albumin*
	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorus*	x	Cholesterol*
	Potassium*	x	Globulins
	Sodium*	x	Glucose*
	Enzymes:	x	Total bilirubin*
x	Alkaline phosphatase	x	Total protein*
	Cholinesterase	x	Triglycerides
	Creatinine phosphokinase*	x	A/G ratio
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
x	Gamma glutamyl transpeptidase		

3) Urinalysis:

Collection times for urine (including # of animals):
6, 12 and 24 months from same animals as blood.

The following CHECKED (X) parameters were examined:

X		X	
x	Appearance*	x	Glucose*
	Volume*	x	Ketones*
x	Specific gravity*	x	Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*		Nitrate
x	Protein*		Urobilinogen

4) Hepatic Mixed Function Oxidase (MFO) and Peroxisomal Beta-Oxidation Analyses

At 3, 6, and 12 months, livers from 6 mice/sex/group were randomly selected from animals scheduled for post-mortem examinations and analyzed for MFO activity. Additional samples taken from the 12 month sacrifice were frozen and subsequently analyzed for hepatic peroxisomal beta-oxidation activity.

i. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations:

All animals.

Animals (groups) sacrificed at the end of the treatment observation period which were subjected to complete gross pathological examinations:

All animals.

j. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination:

Tissues were preserved from all animals. Only liver was examined microscopically from animals scheduled for sacrifice at 3 and 6 months. At 12 months, all tissues listed below examined for controls and high dose; liver, gross lesions and tissue masses were examined for other dose groups. All tissues examined for non-surviving mice in all dose groups.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination:

At 24 months, all tissues examined in controls and high dose groups; liver, kidneys, lungs, testes and tissues with gross changes were examined in other dose groups. Tissues examined from sentinel mice were brain, liver, kidneys, lung, spleen, liver, colon and other tissues with gross changes.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue		Aorta*	xx	Brain*
x	Salivary glands*	xx	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	xx	Spleen*	x	Eyes (optic n.)*
x	Jejunum*	x	Thymus*		Glandular
x	Ileum*		Urogenital	xx	Adrenals*
x	Cecum*	xx	Kidneys*		Lacrimal gland
x	Colon*	x	Urinary bladder*	x	Mammary gland*
x	Rectum*	xx	Testes*	x	Parathyroids*
xx	Liver*	x	Epididymides	x	Thyroids*
x	Gall bladder*	x	Prostate		Other
x	Pancreas*	x	Seminal vesicle	x	Bone*
	Respiratory	xx	Ovaries	x	Skeletal muscle*
x	Trachea*	x	Uterus*	x	Skin
x	Lung*	x	Vaginat	x	All gross lesions and masses
x	Larynx	x	Coagulating gland*		

* Saved at 12 and 24 months only. The coagulating gland, seminal vesicles/prostate glands were collected at necropsy as a single unit.

k. Statistical Analyses: Body weights, feed consumption, clinical chemistry, hematology, urinalysis and organ weights inspected for normality and homogeneity of variance across treatment groups by examining residual plots. Analysis of variance used in assessments for overall treatment effect; group means compared using Duncan's test when significant treatment effect found. Survival distributions compared separately within each sex and also pooled over sex by using both logrank and Wilcoxon tests found in PROC LIFETEST of the Statistical Analysis System (SAS).

B. RESULTS:

1. Dietary Preparation: Samples for week 1 homogeneity analyses as well as samples retained from weeks 1, 2, 4 and those taken from 4 week intervals were analyzed for RH-3866 concentration. The overall average concentrations for each dose level ranged from 92-105% of the theoretical dosages. The average for the 3 concentrations together was 98%. The individual concentrations for each dose level ranged from 11-40 ppm for the 20 ppm dose level, from 57-130 ppm for the 100 ppm dose level and from 280-890 ppm for the 500 ppm dose level. Obviously, the extreme deviations from the theoretical dose levels did not occur very often.

2. Clinical Observations and Mortality: There was no apparent effect of the test chemical on the survival of the test groups. Percent survival at the end of the 24 month treatment period was 50, 47, 44, and 56% for the controls, 20, 100, and 500 ppm groups, respectively for males and 46, 51, 47 and 51% for the females, respectively. No treatment-related signs of clinical toxicity were observed in any of the test groups. The following signs were observed in all groups: red swollen ears, alopecia, arched back and yellow stained anogenital area. According to the authors, some mice showed some common pre-death signs associated with a debilitated state. These signs included ataxia, tremors and lethargy.

3. Body Weight Determinations: No treatment related changes in body weight were observed in any of the test groups. Significantly decreased body weights were observed when compared to controls at individual times in the highest dose group (only once in the mid-dose group in females after the pretest period), but these were not consistent.

4. Food and/or Water Consumption: No dose-related changes in food consumption were observed with any of the treated groups. Sporatic statistically significant increases and decreases in food consumption were observed in all of the treated groups.

5. Ophthalmological Examinations: No treatment-related abnormalities were observed in any of the treated groups.

6. Hematology: No treatment-related changes were observed in any test group. A significant increase in the mean corpuscular hemoglobin concentration value was observed in female rats at the 20 ppm dose level at 6 months. This did not occur in any of the higher dose levels, nor did it occur again at any of the other time periods. Therefore, it was considered to be spurious.

7. Clinical Chemistry: After 3 months of treatment, SGPT values were increased in female mice at 500 ppm. This was considered to be a treatment-related effect since an increase in MFO activity and an increase in liver weights were observed at this time. Other changes observed at this time were considered to be questionable in terms of their biological significance because they were not seen at later time periods and they were not observed with higher dose levels that were tested in a previous study (mouse subchronic feeding). No treatment-related changes were observed in any of the treated groups at any of the other time periods.

8. Urinalysis: No treatment-related effects were observed in any of the test groups.

9. Hepatic Mixed Function Oxidase Assay: After 3 months of treatment with the test chemical, MFO activity was increased in females at 100 ppm and in both sexes at 500 ppm. At this time point, RH-3866 had no effect on hepatic microsomal protein content at any dose level. After 6 months of treatment, MFO activity was increased in both sexes at 500 ppm. At this dose level, hepatic microsomal protein content was increased 28% and 21% in males and females, respectively. After 12 months of treatment, significant increases in MFO activity were observed in 500 ppm females. Hepatic microsomal protein concentration was not affected at any dose level after 12 months. At this time period, RH-3866 had no effect on peroxisomal ^{14}-palmitoyl-CoA oxidase activity.

10. Gross Pathology: No treatment-related gross changes were observed in any of the treated groups at any time period.

11. Organ Weights: At 3 months, absolute and relative liver weights were significantly increased over controls in both male and female mice fed 500 ppm of the test chemical. No treatment-related changes in organ weights were observed at any dose level at either 6 months, 12 months or 24 months. All the changes that were observed were considered to be either spurious or due to the fact that the control weights were exceptionally low.

12. Histopathology:

    a. Nonneoplastic lesions: Treatment-related changes in the liver were observed in male mice at the 500 ppm level after 3, 6, and 12 months of treatment. These changes included increased incidences and severity of centrilobular hepatocytic hypertrophy,

57

Kupffer cell pigmentation, periportal punctate vacuolation and individual hepatocellular necrosis. Following 24 months of treatment, changes in the liver were observed in both sexes at the 500 ppm level. These included increased incidences of focal hepatocellular alterations and multifocal hepatocellular vacuolation. These were not associated with any hypertrophy, hyperplasia or neoplastic proliferations in the liver. No other treatment-related microscopic changes were observed at any dose level. Table I summarizes the liver effects observed.

b. Neoplastic lesions: No treatment-related increases in any neoplasms were observed at any dose level. Hepatocellular hypertrophy and hepatocellular adenomas or carcinomas occurred in mice of all groups, including the controls. RH-3866 had no effect on the severity or incidence of these lesions. Other neoplastic lesions of the liver were also found in all groups: hemangioma, hemangiosarcoma, lymphoreticular lesions, and metastatic or invasive tumors. Adenomas and carcinomas of the lungs were found in all groups as well as lymphosarcomas and other neoplastic lesions of the lymphoreticular system. The attached tables summarize the incidence of neoplastic lesions found. Two tables are given, one for mice which either died or were sacrificed prior to and including 12 months and one for mice which either died or were sacrificed at the termination of the study.

c. Sentinel mice: There was no indication of any intercurrent disease.

13. Quality Assurance Measures: The study was audited and reviewed numerous times by the Quality Assurance Unit for adherence to GLP's and the final report was signed by this group.

C. DISCUSSION: As a chronic feeding study, this appears to be a well conducted study. It is classified as CORE GUIDELINE for a chronic feeding study. The study is classified CORE SUPPLEMENTARY as an oncogenicity study because the Toxicology Branch (TB) does not believe that the top dose level tested was sufficiently high enough. It does not appear that the Maximum Tolerated Dose (MTD) was reached. The effects seen at the highest dose level tested were increases in liver mixed-function oxidase, SGPT and liver weights; and microscopic alterations of the liver consisting of centrilobular hypertrophy, vacuolation, Kupffer cell pigmentation and altered foci (eosinophilic, basophilic, vacuolated and clear cell types). These effects are not considered to be sufficiently severe enough to establish that the highest level tested (500 ppm) approached the MTD.

This chemical was tested in a 3-month dietary subchronic study. The dose levels tested were approximately 3, 10, 30, 100, 300, 1000, 3000 and 10,000 ppm. No effects were observed with dose levels up to and including 300 ppm. At 1000 ppm, increased liver enzyme activity and

liver weights were observed as well as hepatocytic hypertrophy, vacuolation and a borderline count of individual hepatocytic necrosis. At 3000 ppm, in addition to the effects noted above, an increase in SGOT in males (although not statistically significant) and pigmentation of the Kupffer cells were observed. A more significant count of individual hepatocytic necrosis was seen but this was not noted in any of the animals exposed to 10,000 ppm. At 10,000 ppm, increases in liver enzymes, BUN, and kidney weights were noted in addition to the effects observed above as well as some hematological effects. Clearly, at this dose level more significant toxicological effects were being observed. Based upon the effects noted in the subchronic study in mice, TB believes that higher dose levels should have been used in the mouse chronic study.

Summary of Nonneoplastic Liver Effects Observed in Mice After Chronic Exposure to RH-3866

Observed Effect	Dose Level							
	Males				Females			
	0 ppm	20 ppm	100 ppm	500 ppm	0 ppm	20 ppm	100 ppm	500 ppm
Hepatocellular Centrilobular Hypertrophy								
3 months	1/10	1/10	1/10	9/10				
6 months	2/10	2/10	1/10	9/10				
12 months	5/20	6/20	5/20	16/20				
12-24 months	8/66	6/63	5/65	11/62				
Sentinel (12 mo.)	1/5							
Kupffer Cell Pigmentation								
6 months	0/10	0/10	0/10	5/10				
12 months	4/20	1/20	4/20	12/20				
Sentinel (12 mo.)	2/5							
Periportal Punctate Vacuolation								
3 months	0/10	0/10	0/10	2/10	0/10	0/10	0/10	1/10
6 months	0/10	0/10	0/10	3/10	0/10	0/10	1/10	2/10
12 months (multifocal)	0/20	0/20	0/20	4/20	0/20	1/20	1/20	3/20
Sentinel (multifocal)	0/5	1/5						
Individual Hepatocellular Necrosis								
6 months	1/10	1/10	3/10	3/10				
12 months	2/20	1/20	1/20	6/20	0/20	0/20	1/20	2/20
Sentinel (12 mo.)	2/5	0/5						

Summary of Liver Effects (Continued)

Observed Effect	Dose Level							
	Males				Females			
	0 ppm	20 ppm	100 ppm	500 ppm	0 ppm	20 ppm	100 ppm	500 ppm
Focal Hepatocellular Alterations								
focus/foci, basophilic	2/66	3/63	1/65	4/62	0/64	0/66	1/66	2/67
focus/foci, clear-cell	0/66	0/63	0/65	2/62	0/64	0/66	0/66	0/67
focus/foci, eosinophilic	2/66	1/63	4/65	5/62	2/64	2/66	1/66	4/67
focus/foci, vacuolated cell	0/66	0/63	1/65	0/62	0/64	0/66	0/66	0/67
Total incidence	4/66	4/63	5/6/65*	10/11/62*	2/64	2/66	2/66	6/67
Multifocal Hepatocellular Vacuolation								
centrilobular	1/66	1/63	0/65	1/62	0/64	1/66	0/66	0/67
diffuse	0/66	0/63	0/65	0/62	0/64	0/66	0/66	1/67
multifocal	1/66	0/63	2/65	7/62	3/64	2/66	0/66	7/67

* / = number of mice with hepatocellular alteration/actual incidence of hepatocellular alteration. In 4 instances, a mouse had more than 1 type of hepatocellular alteration (or neoplasia, which were not included in this table).

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EPA: 68-02-4225
DYNAMAC No. 1-033-B1,2
January 27, 1986

DATA EVALUATION RECORD

RH-53,866

Subchronic Oral Toxicity Study in Rats

STUDY IDENTIFICATION: O'Hara, G. P. and DiDonato, L. J. Three month dietary toxicity study in rats. (Unpublished study No. 83R-068 prepared by the Toxicology Department, Rohm and Haas Co., Spring House, PA, for Rohm and Haas Co., Philadelphia, PA; dated August 7, 1984.) Accession Nos. 072897-072898.

APPROVED BY:

J. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: J. Cecil Felkner

Date: 1-27-86

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1. CHEMICAL: RH-53,866; RH-3866; alpha-butyl-alpha-4-chlorophenyl-1H-1,2,4-triazole-1-propanenitrile.

2. TEST MATERIAL: RH-53,866 (81.1% active ingredient) from lot No. LSPL00161E, TD No. 83-076, was described as a brown solid.

3. STUDY/ACTION TYPE: Subchronic oral toxicity study in rats.

4. STUDY IDENTIFICATION: O'Hara, G. P. and DiDonato, L. J. Three month dietary toxicity study in rats. (Unpublished study No. 83R-068 prepared by the Toxicology Department, Rohm and Haas Co., Spring House, PA, for Rohm and Haas Co., Philadelphia, PA; dated August 7, 1984.) Accession Nos. 072897-072898.

5. REVIEWED BY:

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Jane Harris, Ph.D.
EPA Reviewer and Section Head

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Date: 01/28/86

7. CONCLUSIONS:

Although mixed function oxidase activity was increased at 300 ppm or greater RH-53,866 in the males and at 1,000 ppm or greater in the females, this is not considered an effect for the purposes of establishing a toxicity NOEL, but rather an indication of the power of accommodation of the liver. The LOEL is considered to be 3,000 ppm (150 mg/kg/day) on the basis of gross changes in the liver, increases in relative and absolute liver and relative kidney weights, and histopathologic changes in the liver and kidneys. Hence, the NOEL for systemic effects is considered to be 1000 ppm (equivalent to an actual intake of 50 mg/kg/day).

Core Classification: Core Minimum.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. The test material was liquefied in a water bath at 60°C, homogenized, dissolved in acetone, mixed with feed in a Hobart mixer, and added to the control diet. The diets were tested for homogeneity, and selected samples were evaluated by an unstated analytical procedure.

2. The test animals, COBS-CD(SD) BR strain rats (90 males and 90 females, 25-28 days old), were received from Charles River Breeding Laboratories, Kingston, NY, and a 4-week quarantine period was observed before the start of dosing. Animals were randomized, stratified by body weight, into dosage groups, each containing 10 males and 10 females, and individual animals were identified with a metal tag bearing a unique number. Animals were housed singly in stainless steel cages in an animal room that was environmentally controlled at 72°F, 40-60% humidity, and a 12-hour light cycle.

3. The diet was Purina Rodent Laboratory Chow (meal) No. 5001. Water and diets were available ad libitum. Groups of 10 rats/sex were fed control diets (designated Group 01) and test diets containing 5, 15, 50, 150, 500, 1,500, 5,000 or 15,000 ppm test material (respectively, Groups 02, 03, 04, 05, 06, 07, 08, and 09). During weeks 3 and 4 doses were increased to 7, 21, 70, 210, 700, 2,100, 7,000, or 21,000 ppm. During weeks 5-13, doses were increased to 10, 30, 100, 300, 1,000, 3,000, 10,000, or 30,000 ppm.

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[1] Only items appropriate to this DER have been included.

4. Animals were observed daily for signs of toxicity, ill health, morbidity, and mortality. Physical examinations were performed weekly. Body weights and feed consumption were measured weekly. Hematologic (7 tests) and clinical chemistry (14 tests) values were measured at 4 and 13 weeks of dosing on all surviving animals. Urinalysis was performed on all males and females of the control and 3,000- and 10,000-ppm dose groups after week 11. Ophthalmoscopic evaluation was conducted at initiation and termination.

5. At termination, survivors were killed and necropsies were performed. Organ weights were recorded for adrenals, brain, gonads, kidneys, liver, spleen, and thyroid/parathyroid (post-fixation). Sections of liver tissues from three rats/sex/dose were taken for determination of aminopyrine N-demethylase and benzphetamine N-demethylase activities in the groups receiving 0, 100, 300, 3,000 and 10,000 ppm RH-53,866. Approximately 35 tissues were taken for histopathologic examination, and these examinations were conducted on the control and 3,000- and 10,000-ppm groups; however, target organs were examined histopathologically at all concentration levels.

6. Normality of distribution of data and homogeneity of variance across groups were assessed by examination of the residual plots. Body weight and food consumption were evaluated by analysis of covariance using pretest values as the covariant.

   Group means were compared using a t-test. Analysis of variance was performed for some parameters on both sexes combined and also on the separate sexes for other parameters. Group means were compared using Duncan's multiple range test.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. Diet Analysis: Analysis of dietary samples for RH-53,866 revealed good approximation between the nominal and analyzed dietary concentrations. Dose levels ranged between 80 and 130% of intended concentrations, and the average concentration of test material was 103% of the nominal.

B. Survival and Clinical Observations: All rats treated with 30,000 ppm RH-53,866 for the last 8 weeks of this 13-week study (Group 09) died during the dosing period, the earliest deaths were after 17 and 18 days of dosing following dosage increase (i.e., during study week 8) in males and females, respectively. Signs of toxicity in those rats included a brown-stained, anogenital area, red or brown muzzle, scant feces, and emaciation. No dose-related deaths occurred at 10,000 ppm or lower; however, one male receiving 10,000 ppm was found dead on day 83. No dose-related signs of toxicity were observed at 10,000 ppm or lower doses.

C. Mean Body Weights and Food Consumption: The parameters were significantly decreased ($p < 0.05$) in both sexes at 30,000 and 10,000 ppm. At 3,000 ppm of RH-53,866, male body weights were decreased from weeks 6-12 without a corresponding decrease in food consumption. Food consumption was significantly decreased at 30,000 ppm of RH-53,866 for both males and females. At 10,000 ppm, food consumption was decreased for the males throughout the dosing period and was decreased for the females for the first 11 weeks of dosing. Table 1 presents selected body weight data.

D. Hematology: The hematologic effects in the 30,000 ppm group (measured at week 4 after dosage change) were considered compromised because of the debilitated condition of the animals. They included increased hematocrits (HCT), increased hemoglobin (HGB) and red blood cell counts (RBCs), decreased white blood counts (WBCs) and platelets, increased segmented neutrophils, and decreased lymphocytes and monocytes. In the 10,000-ppm group, effects included increased platelet counts and decreased mean corpuscular volume (MCV) and hemoglobin values [Table 2 (males) and Table 3 (females)]. The authors considered all of the effects related to the test material; however, the magnitude of the effects were slight and were not considered toxicologically significant.

E. Clinical Chemistry: The clinical chemistry findings are presented in Tables 4 and 5 for the males and females, respectively. Changes were noted at doses of 3,000 and 10,000 ppm; no compound-related effects were noted at 1,000 ppm or below. SGOT activity was significantly reduced in the males at 3,000 and 10,000 ppm. Reduced SGOT values have no biological meaning. SGPT was significantly increased in the males only at the 10,000-ppm dose for both intervals. The increased value at 100 ppm in the females is not meaningful and it does not follow a dose-effect relationship.

Cholesterol levels were generally increased in both sexes at 3,000 and 10,000 ppm. When the sexes were combined for analysis, alkaline phosphatase activity was significantly increased at 10,000 ppm; when the sexes were analyzed separately there was not a significant difference relative to the control level. BUN was elevated at 10,000 ppm in both sexes at both intervals. The serum phosphorus level was significantly decreased in the 10,000-ppm male group and significantly increased in the females; however, the study authors considered this to be of no toxicologic significance. Serum calcium was marginally increased in the 10,000-ppm males only at the 13-week interval. GGT activity was increased in both males and females of the 10,000-ppm group, but only at the 13-week interval. Total protein levels were increased in the 10,000-ppm dose for both sexes. Globulin levels were increased in both sexes at 10,000 ppm and in the males at 3,000 ppm. This was reflected in the A/G ratio, which was reduced in the 10,000-ppm female group when compared to the controls.

TABLE 1. Summary of Selected Mean Body Weights (g) and Food Consumption (g/rat/day) at Selected Intervals for Male and Female Rats Fed RH-53,866 in the Diet for 3 Months

Dose Levels (ppm)	Males			Females		
	Week			Week		
	0	6	13	0	6	13
	Body Weight					
0	294±14	437±23	517±28	184±10	248±18	285±25
100	286±12	423±28	504±45	181± 6	243± 9	282±18
300	296±18	437±27	522±42	182±12	247±20	285±25
3,000	292±16	408±25*	476±28	181±10	239±16	269±20
10,000	298±16	326±35*	365±54*	181±11	226±20*	244±29*
30,000	296±18	175±52*	--	182±12	127±19*	--
	Food Consumption					
0	27.1±1.3	27.0±1.3	25.2±1.0	18.2±1.2	19.8±1.8	17.2±2.0
100	25.9±2.3	26.2±2.0	25.0±3.4	17.8±1.1	20.1±1.3	18.9±1.6*
300	26.8±2.3	26.5±1.5	25.8±2.6	17.4±1.0	19.8±2.0	18.4±1.4*
3,000	26.7±1.3	25.8±2.2	24.9±2.1	17.8±1.2	19.0±1.5	18.4±1.4
10,000	27.7±2.1	21.9±3.4*	23.4±4.7	18.0±0.9	17.2±3.2*	17.7±2.8
30,000	27.3±2.1	14.6±3.8*	--	18.1±2.2	9.4±6.3*	--

*Significantly different from the control value ($p < 0.05$).

TABLE 2. Summary of Selected Mean Hematology Data at Selected Dose Levels Following Administration of RH-53,866 in the Diet of Male Rats for 3 Months

Test	Week	Dose Level 0 ppm	3,000 ppm	10,000 ppm
HCT	46	61.8 ± 4.5	61.2 ± 2.2	58.5 ± 3.9
(%)	13	59.0 ± 7.6	59.2 ± 4.1	57.6 ± 4.4
HGB	4	15.79 ± 0.90	15.58 ± 0.60	14.97 ± 0.96*
(g/100 mL)	13	15.81 ± 1.90	15.91 ± 0.73	15.40 ± 0.66
RBC	4	8.99 ± 0.72	9.12 ± 0.20	8.84 ± 0.67
(10^6/mm^3)	13	9.37 ± 1.33	9.69 ± 0.40	9.97 ± 0.41
Platelet	4	843 ± 124	929 ± 99	972 ± 131
(10^3/mm^3)	13	934 ± 209	906 ± 124	1095 ± 135*
MCV	4	68.4 ± 2.1	67.1 ± 1.8	66.2 ± 1.9
(m^3)	13	63.2 ± 3.8	60.9 ± 3.5	57.9 ± 3.9*
WBC	4	14.6 ± 3.5	16.7 ± 3.9	15.6 ± 3.4
(10^3/mm^3)	13	10.7 ± 2.3	10.3 ± 2.3	11.2 ± 3.9

*Significantly different from control value ($p < 0.05$).

TABLE 3. Summary of Selected Mean Hematology Data at Selected Dose Levels Following Administration of RH-53,866 in the Diet of Female Rats for 3 Months

Test	Week	Dose Level 0 ppm	3,000 ppm	10,000 ppm
HCT (%)	4	59.5 ± 2.9	59.8 ± 2.7	59.9 ± 2.9
	13	58.3 ± 3.0	56.5 ± 3.1	55.3 ± 2.6
HGB (g/100 mL)	4	15.4 ± 0.6	15.2 ± 0.5	15.0 ± 0.7
	13	15.7 ± 0.5	15.3 ± 0.7	14.8 ± 0.5*
RBC (10^6/mm^3)	4	8.48 ± 0.45	8.58 ± 0.42	8.75 ± 0.47
	13	8.71 ± 0.45	8.74 ± 0.42	9.31 ± 0.38
Platelet (10^3/mm^3)	4	884 ± 118	911 ± 139	1009 ± 89*
	13	936 ± 75	933 ± 114	1099 ± 130
MCV (m^3)	4	70.0 ± 0.9	69.7 ± 0.8	68.4 ± 1.4*
	13	66.8 ± 2.5	64.7 ± 4.3	59.6 ± 4.1*
WBC (10^3/mm^3)	4	11.7 ± 5.1	11.7 ± 3.1	14.9 ± 3.9
	13	6.9 ± 1.7	7.3 ± 3.0	7.8 ± 3.0

*Significantly different from control value ($p < 0.05$).

TABLE 4. Summary of Selected Mean Clinical Chemistry Data at Selected Dose Levels Following Administration of RH-53,866 in the Diet of Male Rats for 3 Months

Test	Week	Dose Levels 0 ppm	3,000 ppm	10,000 ppm
SGOT	4	75.6 ± 19.7	63.0 ± 18.1	61.0 ± 20.7
(U/L)	13	107.9 ± 21.1	83.0 ± 18.5*	84.1 ± 22.4*
SGPT	4	22.7 ± 3.1	22.5 ± 4.6	29.2 ± 9.0*
(U/L)	13	26.6 ± 2.6	25.1 ± 6.3	38.8 ± 10.0*
Cholesterol	4	46.4 ± 11.3	59.5 ± 10.4	121.1 ± 14.8*
(mg/dL)	13	58.9 ± 19.3	74.9 ± 11.6*	141.4 ± 24.0*
Creatinine	4	72.3 ± 17.9	70.5 ± 22.8	68.7 ± 27.2
(U/L)	13	53.1 ± 17.3	59.3 ± 15.6	66.4 ± 15.9
BUN	4	14.4 ± 2.0	15.5 ± 2.2	18.6 ± 3.4*
(mg/dL)	13	12.3 ± 1.9	13.1 ± 2.0	18.4 ± 2.0*
Phosphorus	4	8.5 ± 0.7	7.9 ± 0.6	7.4 ± 0.8*
(mg/dL)	13	6.7 ± 0.6	6.4 ± 0.6	6.1 ± 0.4*
CA^{++}	4	11.23 ± 0.53	11.15 ± 0.71	11.28 ± 0.40
(mg/dL)	4	10.55 ± 0.47	10.73 ± 0.63	11.27 ± 0.70*
GGT	4	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 1.5
(U/L)	13	0.0 ± 0.0	0.0 ± 0.0	8.9 ± 4.0*
Total	4	6.16 ± 0.21	6.29 ± 0.28	6.84 ± 0.39*
Protein	13	6.25 ± 0.37	6.32 ± 0.41	6.92 ± 0.6*
Globulin	4	2.72 ± 0.25	2.91 ± 0.30*	3.21 ± 0.33*
(g/dL)	13	2.75 ± 0.48	2.79 ± 0.45	3.14 ± 0.64
A/G	4	1.27 ± 0.14	1.18 ± 0.16	1.14 ± 0.14
Ratio	1	1.33 ± 0.37	1.30 ± 0.27	1.26 ± 0.32

*Significantly different from control value. ($p < 0.05$).

TABLE 5. Summary of Selected Mean Clinical Chemistry Data at Selected Dose Levels Following Administration of RH-53,866 in the Diet of Female Rats for 3 Months

Test	Week	DOSE LEVELS 0 ppm	3,000 ppm	10,000 ppm
SGOT (U/L)	4	75.1 ± 20.4	65.3 ± 12.8	55.7 ± 9.0
	13	87.9 ± 19.6	95.5 ± 23.8	77.1 ± 18.6
SGPT (U/L)	4	24.8 ± 4.5	18.7 ± 3.4	23.8 ± 6.9
	13	32.1 ± 6.3	25.1 ± 6.7	26.3 ± 5.4
Cholesterol (mg/dL)	4	52.4 ± 13.8	87.6 ± 20.2*	132.6 ± 15.8*
	13	64.8 ± 17.2	109.6 ± 11.3*	182.5 ± 25.4*
ALK Phos. (U/L)	4	54.7 ± 16.1	51.3 ± 16.5	55.9 ± 22.8
	13	44.2 ± 16.2	38.1 ± 16.1	58.2 ± 24.6
BUN (mg/dL)	4	15.7 ± 2.8	19.6 ± 2.0	21.5 ± 5.1*
	13	15.0 ± 2.3	17.6 ± 1.8	21.9 ± 2.7*
Phos (mg/dL)	4	7.7 ± 0.7	7.9 ± 0.7	7.6 ± 0.6
	13	5.5 ± 0.9	5.9 ± 0.8	6.5 ± 0.6*
Ca^{++} (mg/dL)	4	11.40 ± 0.32	11.40 ± 0.35	11.76 ± 0.35
	13	11.03 ± 0.31	10.77 ± 0.65	11.13 ± 0.49
GGT (U/L)	4	0.0 ± 0.0	1.8 ± 1.9	2.9 ± 1.4
	13	0.0 ± 0.0	0.4 ± 0.8	9.1 ± 4.3*
Total protein (g/dL)	4	6.52 ± 0.43	6.60 ± 0.68	7.04 ± 0.45*
	13	6.60 ± 0.64	6.85 ± 0.54	7.00 ± 0.64
Globulin (g/dL)	4	2.65 ± 0.33	2.98 ± 0.56	3.39 ± 0.41*
	13	2.47 ± 0.55	3.10 ± 0.59	3.35 ± 0.74*
A/G Ratio	4	1.48 ± 0.25	1.25 ± 0.24	1.10 ± 0.16*
	13	1.77 ± 0.55	1.27 ± 0.38	1.17 ± 0.44*

F. Urinalysis: No alteration in the urinalysis data could be associated with the administration of the test material.

G. Mixed Function Oxidase Studies: RH-53,866 produced increased hepatic mixed function oxidase (MFO) activity (Table 6) in the male rats at 300 ppm and greater and in females at doses of 1,000 ppm and greater. At 10,000 ppm, enzyme activities were increased as much as 6.5- and 8-fold in males and females, respectively. The authors considered these effects compound related, but not adverse toxicologic effects.

H. Ophthalmologic Evaluation: No ophthalmoscopic changes associated with the test material were observed. There were no dose-related patterns in the distribution of ocular abnormalities and no changes were considered related to dosing.

I. Gross Necropsy Findings: The 30,000-ppm dose group exhibited many compound-related effects in addition to those due to the extreme debilitation that occurred prior to death (all dead in 63 days). These effects included reddened lungs, small spleens, dark adrenal glands, red foci, or reddened mucosa of the stomach of both sexes. Small seminal vesicles were apparent in the males. The liver and kidney were dark.

The kidneys were darker than normal in the 10,000-ppm dose group. The liver architecture was prominent or accentuated in the males dosed with 1,000, 3,000, and 10,000 ppm and in the females dosed with 10,000 ppm. Darkened livers were seen in the 3,000-ppm males and in both sexes at doses of 10,000 and 30,000 ppm. Enlarged livers were observed in the males of the 3,000- and 10,000-ppm dose groups, but not in the females or in either sex at 30,000 ppm.

There were other miscellaneous observations occurring at low frequencies that were comparable in the control and dosed groups or which were unrelated to the compound dose.

J. Organ Weights: Significant ($p < 0.05$) changes in absolute and relative organ weights (see Table 7) when compared to control values were as follows:

1. Adrenal absolute weights were decreased in the 3,000- and 10,000-ppm male groups.

2. Brain absolute and relative weights were increased in the 10,000-ppm male group.

3. Relative gonad weights were increased in both males and females at 10,000 ppm.

4. The absolute heart weight for the 10,000-ppm male group was decreased whereas the relative weight was increased. In the females, there was no effect on absolute weights but the relative weights at doses of 3,000 and 10,000 ppm, respectively, were increased.

TABLE 6. Summary of Mean Microsomal Protein Concentration and Hepatic Mixed Function Oxidase (MFO) Activity in Male and Female Rats Fed RH-53,866 for 13 Weeks (Values were determined at termination.)

Dose Group (ppm)	Microsomal Protein (mg/g liver)	Nmol product formed/mg microsomal protein (Nmol/mg) AP[a]	(Nmol/mg) BP[b]	(Nmol/g liver) AP[a]	(Nmol/g liver) BP[b]
			MALES		
0	38.00 ±8.21	10.98 ±1.61	8.04 ±0.89	418.6 ±118.1	308.6 ± 89.2
100	49.04 ±6.34	12.01 ±3.05	7.62 ±1.75	587.4 ±152.6	371.9 ± 85.0
300	54.08*±1.60	14.35 ±2.29	9.86 ±1.51	778.5*±147.1	534.5*± 97.9
1,000	45.52 ±2.28	16.46*±1.47	15.81*±4.19	750.1*± 85.8	722.1*±203.4
3,000	62.64*±0.72	20.00*±2.47	25.09*±0.39	1253.8*±167.5	1570.9*± 10.0
10,000	72.96*±3.75	23.41*±1.80	27.31*±1.37	1712.4*±211.7	1994.8*±185.6
			FEMALES		
0	44.88 ±5.82	6.01 ±1.23	5.17 ±0.88	266.0 ± 31.5	230.1 ± 30.8
100	37.52 ±5.59	6.32 ±0.99	5.20 ±0.81	238.9 ± 63.2	196.2 ± 49.6
300	50.00 ±4.50	8.13 ±1.74	6.50 ±1.76	408.4 ±103.9	326.7 ± 97.0
1,000	47.52 ±4.08	10.75*±1.70	9.57*±1.64	513.7*±117.1	456.0 ±100.7
3,000	48.24 ±2.13	19.46*±1.44	19.94*±1.38	938.1*± 66.9	960.9*± 58.7
10,000	63.60*±8.52	24.48*±2.66	29.07*±5.34	1545.0*±111.0	1831.9*±263.0

[a]AP: aminopyrine N-demethylase.

[b]BP: benzphetamine N-demethylase.

*Significantly different from control value ($p < 0.05$).

TABLE 7. Selected Mean Absolute (Abs) and Relative (Rel) Organ Weights for Male and Female Rats Fed RH-53,866 in the Diet for 3 Months (Absolute values are in grams; relative values are organ weight x 1000/body weight.)

Dose Group (ppm)	Adrenal		Brain		Gonad		Heart		Kidneys		Liver		Spleen		Thyroid	
	(Abs)	(Rel)	(Abs)	(Rel)	(Abs)	(Rel)	(Abs)	(Rel)	(Abs)	(Rel)	(Abs)	(Rel)	(Abs)	(Rel)	(Abs)	(Rel
MALES																
0	0.057	1.20	2.13	44.7	3.36	70.1	1.398	29.2	3.20	67.0	12.18	254	0.684	14.3	0.026	0.5
1,000	0.053	1.12	2.07	43.8	3.53	74.7	1.441	30.4	3.39	71.4	13.63	287	0.672	14.2	0 026	0.5
3,000	0.045*	1.00	2.10	47.4	3.67	82.9	1.375	31.0	3.51	79.0*	15.68*[a]	352*[a]	0.611	13.8	0.029	0.6
10,000	0.044*	1.33	1.96*	59.5*	3.37	102.1*	1.168*	34.5*	3.10	91.9*	19.75*[a]	581*[a]	0.559*	16.6	0.025	0.7
FEMALES																
0	0.067	2.55	1.92	73.2	0.116	4.43	0.818	31.2	1.90	72.1	6.44	246	0.385	14.7	0.021	0.7
1,000	0.067	2.64	1.89	74.2	0.115	4.46	0.815	31.9	1.87	73.1	7.06	276*[a]	0.428	16.7	0.020	0.7
3,000	0.068	2.77	1.88	76.1	0.129	5.22	0.843	33.9*	1.97	79.2*	8.17*[a]	329*[a]	0.437	17.6	0.018	0.7
10,000	0.057	2.57	1.36	84.1	0.138	6.09*	0.765	34.3*	1.81	80.8*	11.30*[a]	504*[a]	0.429	19.1*	0.021	0.9

*Significantly different from control value ($p < 0.05$).

[a]The reviewers calculated significant differences from the control for the liver weights only (absolute and relative) using Duncan's test; the indica values were significant ($p < 0.01$).

5. Relative kidney weights were increased in both sexes at 3,000 and 10,000 ppm.

6. Absolute and relative liver weights were increased in both sexes at 3,000 and 10,000 ppm. In addition, the relative liver weight is also increased in the 1,000-ppm female group.

7. In the spleen, the absolute weight was decreased for the male whereas the relative spleen weight was increased for the female 10,000-ppm dose groups, respectively. Relative thyroid weight was increased in the male groups at 3,000 and 10,000 ppm; for the females in this dose group the relative thyroid weight was also increased. Many of the significant changes in organ-to-body weight ratios were the result of significantly ($p \leq 0.05$) decreased terminal body weight in males and females at 10,000 ppm and in males at 3,000 ppm.

K. Histopathologic Findings: Changes in the liver (Table 8) due to exposure at a dose of 10,000 ppm RH-53,866 consisted of centrilobular to panlobular hepatocellular hypertrophy, vacuolated hepatocytes, hepatocellular necrosis, and hepatocellular coagulation necrosis. Hypertrophy and hepatocellular necrosis occurred in both sexes at 3,000 ppm and above.

Pigmentation of the convoluted tubular epithelium of the kidney occurred at doses of 3,000 and 10,000 ppm in male animals only. This pigment was not positive for hemosiderin. In the male rats at 10,000 ppm as well as in the controls, hemosiderosis did occur in the red pulp of the spleen. Other compound-related histomorphologic changes included vacuolation of the adrenal cortices of both sexes at 3,000 and 10,000 ppm, an increase in small follicles of the thyroid in the 3,000- and 10,000-ppm males, and an increase in frequency of chronic alveolitis in the 10,000-ppm group of both sexes.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. "When fed to male and female rats for 3 months, RH-3866 has a NOEL of 100 ppm in the diet in males and 300 ppm in the diet in females. The only effect seen in males at 300 ppm was an increase in hepatic MFO activity. No adverse effects were seen at doses up to and including 1000 ppm."

B. The study protocol indicated that the study was to be conducted under good laboratory practices. A signed, but undated, quality assurance form was included in the report.

TABLE 8. Summary of Incidence of Liver Lesions in Male and Female Rats Fed RH 3866 for 90 Days

Dose: (ppm)	Control		300		1000		3000		10,000	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals examined:	10	10	10	0	10	10	10	10	10	10
Centrolobular hypertrophy with increased eosinophilia	0	0	0	-	0	0	10	7	10	10
Vacuolated swollen hepatocytes	0	0	0	-	0	0	0	0	9	0
Hepatocellular necrosis	0	0	0	-	0	0	1	3	1	1
Fatty metamorphosis	0	0	0	-	2	1	1	0	0	0
Necrosis, coagulation, zones	1	0	0	-	0	0	0	0	2	0

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The highest dose used in this study, 30,000 ppm, was clearly far in excess of the maximum tolerated level and produced total mortality (during study week 8). For this reason, little further attention will be given to this group. Although all doses referenced in all sections of this DER indicate an array of 0, 10, 30, 100, 300, 1,000, 3,000, or 10,000 ppm, it is important to remember that these doses were only given to the animal for weeks 5-13; for the first 2 weeks, the doses were only half of this level. Doses at weeks 3-4 were intermediate.

There was no effect on mortality and no compound-related clinical signs were observed at doses of 10,000 ppm or below. Body weight, however, was significantly depressed at 3,000 ppm and above in the male groups and at 10,000 ppm and above in the female groups. There was no correlation of growth to food consumption in the 3,000-ppm male groups, but there was body weight suppression that correlated with reduced food consumption in the 10,000- and 30,000-ppm groups of both sexes.

In the 10,000-ppm group, the decreased HGB and HCT counts together with decreased MCV values and increased RBC counts indicated that there was red cell destruction with compensatory red cell production. There was mild hemosiderosis in the spleens of the controls of both sexes and in 3,000 and 10,000 ppm groups. The hemosiderosis was more severe in the high dose males. Nevertheless, the red cell distruction was very slight and of little toxicologic significance with the exception of the high-dose males where hemosiderosis was evident.

The clinical chemical changes were many and varied, and many of these point to an adverse effect on the liver and kidney. Although some of the values were statistically significant, they are considered to be incidental or spontaneous in nature and are likely to be of little toxicological importance for the specific reasons that follow:

1. Reduced SGOT at 3,000 and 10,000 ppm; low values have no pathologic significance.

2. Phosphorus was reduced in the 10,000-ppm male group; however, phosphorus was increased in the 10,000-ppm female group.

3. Increased calcium levels in the 10,000-ppm groups of both sexes were of little toxicologic significance.

Although the authors concluded that only increased cholesterol and GGT were the only toxicologically adverse effects in the 10,000-ppm groups of both sexes, it is our assessment that increased SGPT at weeks 4 and 13 in the 10,000-ppm males, increased BUN at weeks 4 and 13 in the 10,000-ppm groups for both sexes, and increased total protein, globulin, and A/G ratios in these high-dose groups of both sexes were all induced by the test compound and are toxicologically meaningful. Clinical chemistry findings associated with RH-53,866 administration were not evident at doses below 10,000 ppm.

85

Mixed function oxidase activity was increased in males at doses of 300 ppm or greater and at doses of 1,000 ppm or greater in females. Gross necropsy findings were limited to the liver and were more pronounced in the males. They consisted of dark discolorations, enlargement, and/or prominent architecture at 1,000 ppm and above.

Increased absolute and relative liver weights were seen at 3,000 and 10,000 ppm in both sexes and increased relative liver weight occurred in the 1,000-ppm female group. Increased relative kidney weights were present in the 3,000 and 10,000 ppm doses in both sexes. Many of the other relative weight values were a result of decreased terminal body weights and are not toxicologically significant.

Histopathologic changes in the liver consisted of hepatocellular necrosis and hepatocellular hypertrophy at 3,000 ppm and above. At 3,000 or 10,000 ppm, pigmented convoluted tubules of the kidney were observed in males only. The adrenal cortex was vacuolated in both sexes at 3,000 and 10,000 ppm. An increase in small follicles was seen in the male thyroids at 3,000 ppm and above, and chronic alveolities occurred in the 10,000-ppm group of both sexes.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 340-346, and Appendix B, Protocol, CBI pp. 439-457.

Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
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Budd 1/11/88

DATA EVALUATION REPORT

STUDY TYPE: Chronic/Oncogenicity - Mouse (83-5) TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266090

TEST MATERIAL: RH-3866

SYNONYMS: Rally, Systhane, Myclobutanil

REPORT NUMBER: 84R-023

SPONSOR: Rohm & Haas Company, Philadelphia, PA

TESTING FACILITY: Toxicology Dept., Rohm & Haas Company, Spring House, PA

TITLE OF REPORT: RH-3866: Dietary Chronic and Oncogenicity Study in Mice

AUTHOR(S): P.R. Goldman and J.C. Harris

REPORT ISSUED: October 17, 1986

IDENTIFYING VOLUME: Volume 16 of 47

CONCLUSION: The NOEL was 20 ppm and the LOEL was 100 ppm (slight increase in liver mixed function oxidase). Microscopic changes in the liver were evident in both sexes at 500 ppm.

Classification: CORE GUIDELINE for chronic effects and CORE SUPPLEMENTARY for oncogenicity (see discussion).

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-4-chlorophenyl-1H-1,2,4-triazole-1-propanenitrile
Description: red-brown solid
Batch #(s), Other #(s): Sample # 83-260, lot # LAP-0298
Purity: 90.4%
Source: Rohm & Haas

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female Crl:CD®-1(ICR)BR mice
Age: 3 weeks upon receipt
Source(s): Charles River Breeding Labs

3. Procedure:

a. Dietary Preparation (if applicable): Sample was heated to approximately 70°C until liquified. Liquid was stirred to ensure homogeneity, weighed, dissolved in acetone and mixed with feed in a hood to evaporate the acetone.
Frequency of preparation: weekly
Storage conditions: at room temperature, in dark dry area
Stability Analyses: Each week, an extra feed cup for each dietary level was prepared and left on top of the cage rack in the study room for the treatment week, collected and then submitted for analysis in order to verify stability.
Homogeneity Analyses: The first time the diet was prepared, samples from the top, middle and bottom were taken for analysis.
Concentration Analyses: all samples obtained to assess adequacy of mixing and those obtained during first month for quality assurance were analyzed as well as one sample from each dietary concentration per month. Other samples were preserved and sent to analysis group.

b. Basis For Selection of Dosage Levels:

Not stated, but probably based upon results of subchronic study that was conducted.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 24 months		Interim Sac. 3 months		Interim Sac. 6 months		Interim Sac. 12 months	
		male	female	male	female	male	female	male	female
Contr.	0	70	70	10	10	10	10	20	20
1	20	70	70	10	10	10	10	20	20
2	100	70	70	10	10	10	10	20	20
3	500	70	70	10	10	10	10	20	20
4	Sentinel	25	25	-	-	-	-	-	-

d. Clinical Observations and Mortality: Animals observed daily for signs of ill health and reaction to treatment. Physical exams conducted weekly for first 14 weeks and at 2 week intervals thereafter.

e. Body Weight Determinations: weekly

f. Food and/or Water Consumption: weekly

g. Ophthalmological Examinations (if applicable): 12 and 24 months

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condary Reviewer: Edwin Budd
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DATA EVALUATION REPORT

STUDY TYPE: Rat Chronic/Oncogenicity (83-5) TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266081

TEST MATERIAL: RH-3866

SYNONYMS: Myclobutanil, Systhane, Rally

STUDY NUMBER(S): Sponsor's Project No. 85RC-61, Testing Lab Project No. B342

SPONSOR: Rohm and Haas Company, Spring House, PA

TESTING FACILITY: Tegeris Laboratories, Inc. Laurel, MD

TITLE OF REPORT: Chronic Toxicity and Oncogenicity Study with RH 3866 in Rats

AUTHOR(S): T.E. Shellenberger, L.H. Billups, A.S. Tegeris, D.S. Green

REPORT ISSUED: 10/24/86

IDENTIFYING VOLUME: Volumes 7-13 of 47

CONCLUSION: The NOEL for the study is 2.49 mg/kg/day and the LOEL is 9.84 mg/kg/day based upon testicular atrophy in males. No other significant effects were observed in either sex. The overall mean daily consumption was 0, 2.49, 9.84 and 39.21 mg/kg/day for males and 0, 3.23, 12.86 and 52.34 mg/kg/day for females for the controls, low, mid- and high dose groups, respectively. No oncogenic effects were observed.

Classification: CORE GUIDELINE for the chronic portion of the study and CORE SUPPLEMENTARY for the oncogenicity portion of the study (see discussion).

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-4-chlorophenyl-1-H-1,2,4-triazole-propanenitrile
Description: Solid or viscous solid
Batch #(s), Other #(s): TD #83-260, Lot # LAP 0298 (first 15 weeks); TD #84-038, Lot # 83159-7 (weeks 16ff)
Purity: 90.4% a.i.; 91.4% a.i. (due to error in initial labeling from Sponsor, the dietary conc. of second batch calculated from a value of 92.7% a.i.)
Source: Rohm & Haas

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female Sprague-Dawley rats
Age: 6-8 weeks, dosing to begin after 2-3 week acclimatization period.
Weight(s): Approx. 130-140 grams (F-M, 1 week prior to initiation of study)
Source(s): Charles River Breeding Laboratories, Wilmington, MA

3. Procedure:

a. Dietary Preparation (if applicable): Each jar was heated in a water bath until the sample was liquified; the temperature of water bath did not exceed 90°C. The liquified test chemical was stirred and small aliquots were placed in jars until ready to be used. When used, the sample was heated again, weighed, dissolved in acetone and mixed in the feed. The acetone was evaporated off.
Frequency of preparation: weekly
Storage conditions: room temperature
Stability Analyses: 2-week stability test at all dose levels prior to study
Homogeneity Analyses: Pretest analysis. Samples from top, middle and bottom portions of the feed mixer were retained for analyses. Samples were stored in animal room in feeders and samples obtained after 1 and 2 weeks for assay of compound concentration.
Concentration Analyses: Samples collected at each dose level throughout the study and frozen. Analyses for dose level verification were conducted weekly during first four weeks and subsequently from one set of samples every 4 weeks during the remainder of the study.

b. Basis For Selection of Dosage Levels:

Not stated, however, a subchronic feeding study in rats has been conducted in which the NOEL was 1000 ppm and the LEL was 3000 ppm based upon liver and kidney effects.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered (ppm) Weeks 1-2	Weeks 3-4	Weeks 5 to term
1-Control	0	0	0
2	25	35	50
3	100	140	200
4	400	560	800

30 90

Number of Animals Sacrificed:

Test Group	Main Study 24 months male	female	Interim Sac. 3 months male	female	Interim Sac. 6 months male	female	Interim Sac. 12 months male	female	Interim Sac. 17 months male	female
1	all surviving		10	10	10	10	20	20	18	10
2	all surviving		10	10	10	10	20	20	18	10
3	all surviving		10	10	10	10	20	20	18	10
4	all surviving		10	10	10	10	20	20	18	10

A sentinel animal program was also used with this study. Thirty male and 30 female rats were used. Prior to the initiation of the study, 5 animals of each sex were subjected to a complete viral and microbiological evaluation. At 3, 6 and 12 months, blood sera and/or live animals were submitted to the diagnostic laboratory for evaluation.

d. Procedures for Studies Other Than Feeding and/or Additions, Changes in Feeding Study: Dietary levels were adjusted on the basis of active ingredient content of the test material. Dietary levels were also adjusted during the initial 5 weeks of the study in order to provide a more nearly equal compound intake, mg/kg/day, during the active growth period of the animals.

e. Clinical Observations and Mortality: Twice daily. Detailed examinations when body weights were measured.

f. Body Weight Determinations: -1 weeks, 0, weekly during first 14 weeks, 1x every two weeks thereafter.

g. Food and/or Water Consumption: -1 weeks, weekly during first 14 weeks, 1x every two weeks thereafter.

h. Ophthalmological Examinations (if applicable): Prior to 12-month and terminal necropsies. Performed on all controls and high dose animals. Will be conducted on other animals if effects noted in high dose animals.

91 91

i. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals):
10 males, 10 females/group at 3, 6, 12, 17 months and prior to termination of study.

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	x	Mean corpustular HGB (MCH)
x	Hemoglobin (HGB)*	x	Mean corpustular HGB conc.(MCHC)
x	Leukocyte count (WBC)*	x	Mean corpustular volume (MCV)
x	Erythrocyte count (RBC)*	x	Red cell morphology#
x	Platelet count*		
	Total plasma protein (TP)		
x	Leukocyte differential count*#		

Evaluated only on control and high-dose groups at 6 and 12 months and in all dose groups at 3 months.

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X	Electrolytes:	X	Other:
x	Calcium*	x	Albumin*
	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorus*	x	Cholesterol*
	Potassium*	x	Globulins
	Sodium*	x	Glucose*
	Enzymes:	x	Total bilirubin*
x	Alkaline phosphatase	x	Total protein*
	Cholinesterase	x	Triglycerides#
	Creatinine phosphokinase*	x	A/G ratio
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
x	Gamma glutamyl transpeptidase (GGTP)		

12-month and terminal sacrifice only

3) Urinalysis:

Collection times for urine (including # of animals):
Control and high-dose groups designated for hematology and clinical chemistry collected at 3 (all dose groups), 5, 11, 17 months.

The following CHECKED (X) parameters were examined:

X		X	
x	Appearance*	x	Glucose*
	Volume*	x	Ketones*
x	Specific gravity*	x	Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*		Nitrate
x	Protein*		Urobilinogen

j. Liver Enzyme Assays: Sections of liver from 6 males and 6 females in the control, low, mid and high-dose groups were obtained at the 3, 6 and 12-month sacrifices for determination of mixed function oxidase (MFO) activity. MFO activity was measured by the in vitro enzyme assay of demethylation of aminopyrine (AP). At the 12-month necropsy, livers from 5-6 males and females randomly selected from each group were collected and analyzed for peroxisomal beta-oxidation activity utilizing ^{14}C-palmitoyl-CoA as substrate.

k. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations:

10/sex/group at 3 and 6 months; 20/sex/group at 12 months; 18 males and 10 females at 17 months. All animals found dead or sacrificed in a moribund condition.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations:

All animals.

l. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination:

All animals for liver, testes and ovaries; lungs and kidney: all animals at 12 and 17 months; all organs at 12 months in control and high-dose groups and target organs in mid- and low-dose groups; all organs in animals that were found dead and sacrificed moribund; gross lesions and masses: control and high-dose males and females at 3, 6 and 17 months; all animals at 12 months, all animals that died or were sacrificed moribund.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination:

Liver, testes, ovaries, lungs, kidneys, gross lesions and masses in all animals. Otherwise, all tissues required by protocol in control and high-dose groups and target organs in mid and low-dose groups. In addition, other tissues not required by protocol occasionally were inadvertently examined in some males and females at the 12 and 17-month interim and terminal sacrifices.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

X		X		X	
	Digestive system		**Cardiovasc./Hemat.**		**Neurologic**
	Tongue		Aorta*	xx	Brain*
x	Salivary glands*	xx	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	xx	Spleen*	x	Eyes (optic n.)*
x	Jejunum*	x	Thymus*		**Glandular**
x	Ileum*		**Urogenital**	xx	Adrenals*
x	Cecum*	xx	Kidneys*		Lacrimal gland
x	Colon*	x	Urinary bladder*	x	Mammary gland*
x	Rectum*	xx	Testes*	x	Parathyroids*
xx	Liver*	x	Epididymides	x	Thyroids*
	Gall bladder*	x	Prostate		**Other**
x	Pancreas*	x	Seminal vesicle	x	Bone*
	Respiratory	xx	Ovaries	x	Skeletal muscle*
x	Trachea*	x	Uterus*	x	Skin
x	Lung*			x	All gross lesions and masses
x	Larynx				

m. Statistical Analyses: one-way Analysis of Variance followed by Dunnett's t-test. Percent survival estimated with Lifetest Procedure (SAS Institute).

B. RESULTS:

1. Dietary Preparation: Measured dose levels ranged between 83-108% of the desired levels. The average was 95%.

2. Clinical Observations and Mortality: Treatment with the test chemical did not affect the survival of males or females at any dose level. No difference in mortality was noted. By week 105 the total mortalities in males were 35, 35, 32 and 30 in the control, low, mid and high-dose groups, respectively, and 37, 39, 40, and 35 in females respectively. There were no clinical signs observed

that appeared to be related to treatment. The most common clinical signs that were observed throughout the study in all groups, including controls were dermal alopecia, rough haircoat, rash, footpad swelling and mechanical injuries.

3. Body Weight Determinations: The mean weekly body weights of the treated males were similar to controls during the first 8 weeks of the study. After that time, the mean weekly body weights of the high dose males began to decline relative to the control values and by week 22, they were significantly less than controls. This continued up to week 40. Although the body weights of the high dose males were statistically significantly less than the controls, the values still remained within 95-97% of the control values. After that time, the mean body weights of the high dose males were always less than controls, but were only significantly less at weeks 56, 80, 82 and 84. The data suggest that the test chemical induced a decrease in the mean body weights of the males in the high dose group when compared to controls between 6 and 18 months. The body weights at the lower dose levels were generally slightly lower than controls during this time period, but the lower values were not considered to be biologically significant. For females, the test chemical appeared to have no effect on the body weights of the treated animals during the first year of the study. During the second year, the test chemical appeared to have a marginal effect on the body weights of the high dose females relative to controls. The body weights were generally lower than controls during weeks 54 to 96 and the differences were statistically significant at weeks 66-72, 76-84 and 92. During weeks 76-84, the body weights were generally between 88-90% of the control values.

4. Food and/or Water Consumption: For males, food consumption was generally lower in the high dose animals when compared to controls, starting around the fifth week. Food consumption was statistically significantly lower for fifteen weeks within the fifth to seventy-eighth week; however, the values never dropped below 91% of the control values. Beginning at week 80, food consumption was similar to controls. No significant differences were noted for the two lower dose male groups. For females, in general, no significant changes in food consumption were observed between the treated and control animals. Based upon the food consumption values, the overall mean daily compound consumption for males was 0, 2.49, 9.84 and 39.21 mg/kg/day and for females was 0, 3.23, 12.86 and 52.34 mg/kg/day for controls, low, mid- and high dose groups, respectively.

5. Ophthalmological Examinations: There were no indications of compound-related ocular abnormalities. The most prevalent abnormality observed prior to termination of the study was diagnosed as conjunctivitis secondary to infectious diseases or dental abnormality.

6. Hematology: No treatment-related differences were observed in any of the treated groups when compared to controls.

95 95

7. Clinical Chemistry: There were a number of occurrences of statistically significant differences in several clinical chemistry values. However, for various reasons, each one of these were considered to be biologically insignificant. Therefore, there were no treatment related changes observed in any of the clinical chemistry parameters.

8. Urinalysis: No consistent differences were noted between the treated and control groups.

9. Hepatic Enzyme Assays: A slight increase in hepatic mixed function oxidase (MFO) activity (24-47%) was seen in high-dose level males at 3, 6 and 12 months. The increases were statistically significant at 3 and 6 months. MFO activity was significantly increased in females in both mid- and high dose groups (61% and 78%, respectively) at 3 months. At 6 and 12 months, slight increases in high dose females were observed but were not statistically significant. RH-3866 had no effect on hepatic peroxisomal ^{14}C-palmitoyl-CoA oxidase activity in rats at dose levels up to 800 ppm after 12 months of dietary treatment.

10. Gross Pathology: The distributions and incidences of palpable masses in both males and females, as well as the mean "time-to-tumor" for the masses indicate that the presence of the masses were unrelated to treatment. The number of animals in each dose group with palpable masses was 1, 7, 2 and 4 in males for the first 12 months; 15, 13, 16 and 9 in males for months 13 to termination; 7, 9, 13 and 2 in females for the first 12 months; and 47, 46, 38 and 39 in females for months 13 to termination in control, low, mid- and high-dose groups respectively.

All gross lesions observed at 3 and 6 months were considered to be unrelated to treatment. This was also true for the gross lesions observed at 12 months except for testicular lesions. Testicular reduction in size was seen in 0, 1, 1 and 3 animals in the control, low, mid and high dose animals, respectively. The higher incidence observed in the high dose animals correlates with the reduction in testicular weights in this dose group. At 17 months, again, only the testicular effects were considered to be related to treatment with the test chemical. Bilateral reduction in testicular size was observed in 2, 2, 0 and 6 animals in the control, low, mid and high dose groups, respectively. At terminal sacrifice, with the exception of the testicular effects, all observed lesions appeared to be unrelated to treatment. Decrease in testicular size was seen in 0, 2, 7 and 6 males respectively, in controls, low, mid- and high dose animals. In addition, a second testicular lesion characterized as "soft" was also seen in 1, 1, 5, and 5 males in the controls, low, mid- and high dose groups, respectively. Reduction in testicular size was also seen in the treated animals that died prior to the completion of the study (2, 6, 2 and 7 in controls, low, mid- and high dose animals, respectively).

11. Organ Weights: In males, no significant differences were observed between mean liver weights and between mean liver-to-body weight ratios in the treated animals versus the controls at any of the sacrifice times. In females, the mean liver-to body-weight ratio in the high dose animals was significantly higher than controls at 3 months (113%) and the mean liver weights in the high dose animals were significantly higher than controls at 6 months (120%). At other times there were increases in liver weights and liver-to-body weight ratios in one or more of the treated groups, but none were statistically significant. The authors state that these results suggest that there may be a marginal effect of the chemical on mean hepatic weights in female rats.

At 12 months, the mean testicular weights and testes-to-body weight ratios in all groups of the treated animals were lower than controls, but only the mean testicular weights in the high dose animals were statistically significant (88% of controls). At 17 months, none of the means for the treated animals were statistically significant, but the means were slightly lower than controls for high dose males (88% and 90% for testes and testes-to-body weight ratio, respectively). At termination, the mean testicular weights of both mid- and high dose groups (77% and 75% of controls, respectively) and the mean testes-to-brain weights of the high dose group were significantly lower than controls (79% of controls). The results suggest an effect at the high dose for testicular weights and a possible marginal effect at the mid-dose. No effects were observed in any of the other organ weights. The changes observed were considered to be random occurrences.

12. Histopathology:

a. Nonneoplastic lesions: With the exception of testicular lesions, all nonneoplastic microscopic findings in the study were considered to be incidental to treatment. Incidental lesions were normally found in the liver, kidneys, lung, heart, spleen, adrenals, pancreas, and thyroid gland. The incidences of unilateral and bilateral testicular atrophy are summarized in Table I. The incidence was similar between control and low dose animals, but was increased in mid- and high dose animals. Microscopically, the seminiferous tubules were frequently devoid of spermatid formation and germinal epithelial cells. In several cases, only Sertoli cells remained. In addition to atrophy, microscopic findings included polyarteritis, periarteritis, mineralization of arterioles and occasionally a sperm granuloma, an interstitial cell tumor, scrotal varicocele, orchitis, oligospermatogenesis and bilateral seminiferous tubule atrophy.

b. Neoplastic lesions: No neoplastic lesions were observed that were considered to be related to treatment. Neoplastic lesions were generally observed either in low incidence in all groups, including controls or only in an occasional animal. Tumors that were seen included hepatocellular adenomas; pituitary adenomas

and chromophobe adenomas; mammary gland adenomas, adenocarcinomas and fibroadenomas and islet cell adenomas of the pancreas (see Table II).

13. Quality Assurance Measures: Quality assurance measures were followed and the study was audited on a monthly basis. The report was signed by the Quality Assurance Manager.

C. DISCUSSION: There were several places in which the procedures in this study deviated from the EPA Testing Guidelines: 1) For the clinical chemistry studies, the same animals were not used for each time point. These animals were sacrificed at each time point. 2) Histopathology on gross lesions in the low and mid-dose animals sacrificed at 3, 6 and 17 months was not conducted. Microscopic examinations were conducted on gross lesions in these dose groups at the 12 month sacrifice, at termination and on all animals that either died or were sacrificed in extremis during the study. 3) The lungs and the kidneys were not examined in the low and mid-dose groups at the 3 and 6 month sacrifice times.

Point number 1 is not considered to be one that would significantly affect the outcome of the study. Since microscopic examinations were conducted on gross lesions and the specific organs mentioned above in point 3 from both the low and mid-dose groups at other sacrifice times, and since the results were negative except for testicular effects, these points are also not considered to be significant to the outcome of this study. Therefore, this study is classified as CORE GUIDELINE for the chronic portion of the study. The NOEL for the study is 2.49 mg/kg/day based upon testicular atrophy in males and the LOEL is 9.84 mg/kg/day. In females, a significant increase in hepatic mixed function oxidase activity was observed in both the mid- and high dose groups at 3 months, the mean liver-to body-weight ratio was elevated at 3 months and the mean liver weight was elevated at 6 months. These effects are most likely an adaptive response. Therefore, it appears that the chemical has a minimal effect on females at the dose levels tested.

The chemical was not oncogenic under the conditions of the study. The study is classified as CORE SUPPLEMENTARY for the oncogenic portion of the study because the Toxicology Branch (TB) does not believe that the top dose level tested was sufficiently high enough. It does not appear that the Maximum Tolerated Dose (MTD) was reached. Other than testicular atrophy, there was a marginal effect on liver weights in females at the highest dose level tested. Body weights were marginally decreased in males, but food consumption was also less than controls. In addition, an increase in liver mixed function oxidase was also observed. These effects are not considered severe enough to establish that the highest dose level tested (800 ppm) approached the MTD. Testicular atrophy is not likely to be life-threatening, and thus, higher dose levels could have been used. In addition, this lesion did not appear in the rat subchronic study. The dose levels selected for the chronic study appeared to be selected on the basis of increase in liver weights and liver enzyme induction.

The dose levels selected for the rat subchronic study were approximately 10, 30, 100, 300, 1000, 3000, 10,000 and 30,000 ppm. No effects were observed with dose levels up to and including 100 ppm. At 300 ppm, increases in liver mixed function oxidase activity were observed. At 1000 ppm, increases in the mean relative liver weights in females and accentuated liver architecture (seen grossly but nothing was noted in the microscopic examinations) were observed in addition to what was seen at 300 ppm. At 3000 ppm, increases in kidney and liver weights and SGOT were observed as well as that noted for 1000 ppm. In addition, hepatocellular hypertrophy was seen in the majority of the animals and hepatocellular necrosis was seen in 1/10 males and 3/10 females. Pigmentation of the convoluted tubular epithelium of the kidney was also observed at this dose level in males only. At 10,000 ppm, the effects noted at 3,000 ppm were observed (hepatocellular necrosis was seen in only 1 animal of each sex) as well as vacuolated swollen hepatocytes, blood effects (indications of red cell destruction and compensatory red cell production and hemosiderosis), increases in liver enzymes and BUN, Kupffer cell pigmentation. All of the animals which received 30,000 ppm died prior to the end of the testing period. Based upon the effects noted in the subchronic study in rats, TB believes that higher dose levels should have been used in the rat chronic/oncogenicity study.

TABLE I
INCIDENCE OF UNILATERAL AND BILATERAL TESTICULAR ATROPHY

	Control	Low	Mid	High
		12-Month Sacrifice		
Bilateral	0/20	0/19	1/20	3/20
Unilateral	0/20	1/19	0/20	0/20
		17-Month Sacrifice		
Bilateral	2/18	2/18	0/18	4/18
Unilateral	2/18	2/18	0/18	1/18
		Terminal Sacrifice		
Bilateral	2/17	1/19	5/20	12/22
Unilateral	2/17	3/19	6/20	2/22
		Animals That Died or Were Sacrificed Moribund		
Bilateral	1/35	4/35	10/32	12/30
Unilateral	6/35	4/35	5/32	5/30
		Total Incidence of Testicular Atrophy Across All Groups*		
Bilateral	5/110	7/110	16/110	31/110
Unilateral	10/110	10/110	11/110	8/110

* Including 3 and 6 month sacrifices (10 animals apiece, except low dose at 3 months had only 9 animals).

Table II. Incidence of Neoplastic Microscopic Findings (Summary of Significant Lesions)

Organ (Lesion)	Males				Females			
	Control	Low	Mid	High	Control	Low	Mid	High
12-Month Sacrifice								
Liver								
Hepatocellular Adenoma	0/20	0/19	0/20	0/20	0/19	0/20	0/20	2/20
Pituitary								
Adenoma	0/20	1/18	0/20	0/19	4/19	3/20	3/20	3/20
Mammary Gland								
Adenocarcinoma	0/1	0/1	0/0	0/4	4/10	2/3	2/5	2/17
17-Month Sacrifice								
Pituitary								
Chromophobe Adenoma	5/6	0/0	0/0	3/3	2/3	0/0	0/0	0/0
Adenoma	1/6	0/0	0/0	0/3	1/3	0/0	0/0	0/0
Mammary Gland								
Adenoma	0/1	0/0	0/0	0/0	2/5	0/0	0/0	1/3
Terminal Sacrifice								
Liver								
Hepatocellular Adenoma	0/17	1/19	0/19	0/22	0/24	0/20	0/20	1/25
Pancreas								
Islet Cell Adenoma	3/17	0/5	0/3	2/22	1/24	1/13	0/13	1/25
Pituitary								
Chromophobe Adenoma	12/17	1/5	3/4	7/22	14/23	18/19	10/15	18/25
Mammary Gland								
Adenocarcinoma	0/2	0/1	0/0	0/3	1/23	0/15	1/11	0/25
Fibroadenoma	0/2	1/1	0/0	0/3	10/23	11/15	6/11	11/25
Adenoma	0/2	0/1	0/0	0/3	3/23	6/15	4/11	2/25

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DATA EVALUATION REPORT

STUDY TYPE: Chronic Feeding Nonrodent (Dog) (83-1)

TOX. CHEM. NO.: 723K

ACCESSION NUMBER: 266088

TEST MATERIAL: RH-3866

SYNONYMS: Systhane, Rally, Myclobutanil, RH-53,866

REPORT NUMBER: 84R-078

SPONSOR: Rohm & Haas Co., Philadelphia, PA

TESTING FACILITY: Rohm & Haas Co., Toxicology Dept., Spring House, PA

TITLE OF REPORT: RH-3866: One Year Dietary Study in Beagle Dogs

AUTHOR(S): P.R. Goldman, J.C. Harris and J.D. Frantz

REPORT ISSUED: October 15, 1986

IDENTIFYING VOLUME: Volume 14 of 47

CONCLUSION: NOEL 100 ppm (3.09 mg/kg/day for males and 3.83 mg/kg/day for females) based upon hepatocellular hypertrophy. Supporting effects in organ weights and clinical chemistry observed. LOEL 400 ppm (14.28 mg/kg/day for males and 15.68 mg/kg/day for females)

Classification: CORE MINIMUM because full histopathology examinations were not submitted on the mid- and low dose levels.

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: alpha-butyl-alpha-4-chlorophenyl-1-H-1,2,4-triazole-propanenitrile
Description: white solid
Batch #(s), Other #(s): Lot No. 83159-7, Sample No. (TD No.) 84-063
Purity: 91.4%
Source: Rohm & Haas

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): male and female beagle dogs
Age: 5 months at start of test
Source(s): Marshall Research Animals (North Rose, NY)

102

102

3. Procedure:

a. Dietary Preparation (if applicable): RH-3866 was heated until liquified, stirred, weighed, dissolved in acetone and blended with the feed. The acetone was evaporated off.

Frequency of preparation: weekly

Storage conditions: Stored at room temperature in jars (one jar per weekly diet preparation)

Stability Analyses: a sample from each weekly preparation was taken for analysis. Select samples were analyzed.

Homogeneity Analyses: The first time the diets were prepared, samples were taken from the top, middle, and bottom of each dietary concentration and submitted for analysis.

Concentration Analyses: This was done in connection with the stability and homogeneity analyses.

b. Basis For Selection of Dosage Levels:

Doses selected on the basis of a one-month range-finding study.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 12 months male	female
Contr.	0	6	6
1	10	6	6
2	100	6	6
3	400	6	6
4	1600	6	6

d. Procedures for Studies Other Than Feeding and/or Additions, Changes in Feeding Study: Control and test diets were offered for same two hours per day.

e. Clinical Observations and Mortality: Dogs were observed daily for clinical signs of toxicity. Physical exams conducted weekly for first month and biweekly for remainder of treatment period.

f. Body Weight Determinations: Weekly

g. Food and/or Water Consumption: daily. Mean feed consumption per group calculated weekly.

h. Ophthalmological Examinations (if applicable): Conducted during pre-test period and during weeks 26 and 52 of treatment period.

i. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals):
weeks -2, -1, 13, 25, 39 and 53

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	x	Mean corpuscular HGB (MCH)
x	Hemoglobin (HGB)*	x	Mean corpustular HGB conc.(MCHC)
x	Leukocyte count (WBC)*	x	Mean corpustular volume (MCV)
x	Erythrocyte count (RBC)*	x	Red blood cell morphology
x	Platelet count*		
	Total plasma protein (TP)		
x	Leukocyte differential count*		

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X	Electrolytes:	X	Other:
x	Calcium*	x	Albumin*
	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorus*	x	Cholesterol*
	Potassium*	x	Globulins
	Sodium*	x	Glucose*
	Enzymes:	x	Total bilirubin*
x	Alkaline phosphatase	x	Total protein*
	Cholinesterase		Triglycerides
	Creatinine phosphokinase*	x	A/G ratio
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
x	Gamma glutamyl transpeptidase		

3) Urinalysis:

Collection times for urine (including # of animals):
On each of 2 consecutive days at weeks -4 (all animals), 25 and 51 weeks (controls and high dose)

The following CHECKED (X) parameters were examined:

X		X	
x	Appearance*	x	Glucose*
	Volume*	x	Ketones*
x	Specific gravity*	x	Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*		Nitrate
x	Protein*		Urobilinogen

j. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations:

None were sacrificed and none died prior to completion of the study.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations:

All animals in all dose groups.

k. Histopathology:

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination:

All animals from high dose and control groups; liver, gallbladder and testes in all dogs from all dose groups. Tissues were preserved from all animals from all dose groups for possible future examination.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	x	Aorta*	xx	Brain*
x	Salivary glands*	xx	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*
x	Stomach*	x	Lymph nodes*	xx	Pituitary*
x	Duodenum*	xx	Spleen*	x	Eyes (optic n.)*
x	Jejunum*	x	Thymus*		Glandular
x	Ileum*		Urogenital	xx	Adrenals*
x	Cecum*	xx	Kidneys*		Lacrimal gland
x	Colon*	x	Urinary bladder*	x	Mammary gland*
x	Rectum*	x	Testes*	xx	Parathyroids*
x	Liver*		Epididymides	xx	Thyroids*
x	Gall bladder*	x	Prostate		Other
x	Pancreas*	x	Seminal vesicle	x	Bone*
	Respiratory	x	Ovaries	x	Skeletal muscle*
x	Trachea*	x	Uterus*	x	Skin
x	Lung*			x	All gross lesions and masses

l. Statistical Analyses: Distributions of all continuous data were inspected for normality and homogeneity of variance across treatment groups. Analyses of variance were used when needed. Duncan's multiple range test was used on some data as well as T-tests.

m. RESULTS:

1. Dietary Preparation: Average dose levels ranged between 97-111% of the theoretical dose levels, with an overall average of 103%.

2. Clinical Observations and Mortality: No deaths were observed and no clinical signs of toxicity were noted at any of the dose levels throughout the study.

3. Body Weight Determinations: The body weights of male dogs at the highest dose level were significantly decreased following one week of treatment but were similar to control values throughout the remainder of the study. The mean body weights of female dogs at this dose level were significantly less than the control values for the first 5 weeks of the study. No treatment related changes were noted in the body weights of either sex at any of the other dose levels.

4. Food and/or Water Consumption: Food consumption of female dogs fed the highest dose level was consistently below that of the controls throughout the study. Food consumption of males at the highest dose level was below that of the controls during the first week but was similar to controls during the remainder of the study. No differences were observed in the food consumption of the other groups when compared to controls.

5. Ophthalmological Examinations: No ophthalmological abnormalities were seen in any of the treated dogs.

6. Hematology: A slightly decreased number of red blood cells (RBC), an increased number of platelets, an increase in mean cell hemoglobin and an increase in the mean corpuscular volume were observed in male dogs at the highest dose level throughout the treatment period. A slight increase in the mean cell hemoglobin was observed in male dogs at 400 ppm. No other treatment related changes (other than spurious differences) were observed in any of the other parameters or in any of the other dose groups.

7. Clinical Chemistry: Increases in inorganic phosphorus and alkaline phosphatase and a decrease in serum albumin were observed in both sexes at the highest dose level. Alkaline phosphatase was also increased in females at 400 ppm. SGPT was increased in males and GGT was increased in females at the highest dose level (1600 ppm). These changes were consistent throughout the treatment period. No other consistent changes were noted in any of the other parameters or in any of the other dose groups.

8. Urinalysis: No treatment related changes were noted in any of the treated groups.

9. Gross Pathology: Gross changes were observed in the livers of high dose dogs of both sexes. These changes consisted of enlargement and/or accentuated lobular architecture (1 male and 3 females). Other changes were considered incidental and were found in all groups, including controls. Frequent changes included reddened portions of the intestinal tract, thickened and reddened mammary glands (females only) and distended uteri (females only).

10. Organ Weights: Increased absolute and relative liver weights were observed in both sexes at 1600 ppm and in females at 400 ppm. No other observed changes in organ weights were considered to be related to treatment. The statistically significant increase in absolute liver weight of 100 ppm female dogs is considered to be due to the larger size of the dogs in this group when compared to control dogs and the statistically significant increase in relative liver weights in the 10 ppm dogs is considered to be due in part to the smaller size of the dogs at this dose level.

11. Histopathology:

a. Nonneoplastic lesions: Compound-related nonneoplastic lesions were observed in the livers of both sexes of dogs from the 400 ppm and from the 1600 ppm dose groups. These lesions included minimal to mild hepatocellular hypertrophy in 1/6 of the 400 ppm male dogs and in 5/6 of the 1600 ppm male dogs and mild to moderate hepatocellular hypertrophy in 2/6 of the 400 ppm female dogs and in 6/6 of the 1600 ppm female dogs. The hypertrophy was characterized by cells with large amounts of pale, eosinophilic, finely granular cytoplasm; in a few more severely affected livers it was noted to be almost panlobular in distribution. "Ballooned" hepatocytes or expanded hepatocytes with large clear cytoplasmic spaces, sometimes containing only a few strands of pink cytoplasm were sporatically observed in some of the more severely affected high dose females. These cells were thought to represent severely hypertrophied, possibly degenerating, hepatocytes. No changes in the liver related to treatment were noted in any of the animals in the lower dose levels. Table 1 summarizes the changes noted in the livers of the treated animals. No treatment related changes were observed in the testes of any of the male animals. Incidences of lesions in treated animals were similar to controls at all dose levels. Changes in other organs were considered to be incidental and not related to treatment.

b. Neoplastic lesions: No neoplastic lesions were observed in any of the animals.

12. Quality Assurance Measures: The report and the original data from the study were reviewed for adherence to the GLP's and the study was audited a number of times throughout the pretreatment and treatment phases. The report is signed by the Quality Assurance Unit.

C. DISCUSSION: This study is in general well conducted. There is, however, one important item missing: a full microscopic examination was not conducted on the mid- and low dose animals. Full microscopic examinations on all animals in nonrodent studies are required by the EPA Guidelines. In addition to incomplete microscopic examinations, there were a few minor missing items in the hematology summary (no mention of the statistically significant increases in mean cell hemoglobin in the top two dose levels and an increase in mean corpuscular volume at the top dose level). The study is CORE MINIMUM because complete microscopic examinations were not submitted on the mid- and low dose levels. In this case, the study was excepted because no other effects were seen in any of the other chronic studies except testicular effects and the testes were examined microscopically at all dose levels in the study. It is not likely that complete microscopic examinations at all dose levels would change the outcome of the study. The NOEL is 100 ppm (3.09 mg/kg/day for males and 3.83 mg/kg/day for females) based upon hepatocellular hypertrophy and the LOEL is 400 ppm (14.28 mg/kg/day for males and 15.68 mg/kg/day for females). Supporting effects were observed as well.

RH-3866: ONE YEAR DIETARY TOXICITY STUDY IN BEAGLE DOGS
PROTOCOL NO. 84P-203 REPORT NO 84R-078

Table 1 Summary Of Histopathology Observations

	Males					Females				
Group:	1	2	3	4	5	1	2	3	4	5
RH-3866 Technical: ppm	0	10	100	400	1600	0	10	100	400	1600
Number of Animals Per Group	6	6	6	6	6	6	6	6	6	6
Tissue/Microscopic Observations										
Liver										
Number Examined	6	6	6	6	6	6	6	6	6	6
Number Normal	3	1	2	4	0	1	2	2	1	0
hepatocellular hypertrophy, centrilobular	-	-	-	1	5	-	-	-	2	6
congestion	1	-	-	-	1	-	-	-	-	-
pigmentation, multifocal	1	1	-	-	-	-	-	-	-	-
mononuclear cell infiltrate, periportal, multifocal	2	-	1	-	-	2	-	-	2	1
eosinophils, periportal, multifocal	1	-	-	-	-	-	-	-	-	-
mononuclear cell infiltrate, central vein, multifocal	1	1	3	-	1	-	3	-	1	1
hepatic cord atrophy, subcapsular	1	-	-	-	1	-	-	-	-	-
mononuclear cell infiltrate, parenchymal, multifocal	-	5	3	1	-	4	2	4	4	5
hepatitis, acute, multifocal	-	-	-	-	-	-	-	-	-	1
"ballooned" hepatocytes, centrilobular	-	-	-	-	-	-	-	-	-	4
vacuolization, periportal, multifocal	-	-	-	-	-	-	-	-	1	-

B. One-Liners

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
90-day feeding - mouse; Rohm & Haas/83R-136; 10/08/86	Technical 81% pure	266079	NOEL=300 ppm, LOEL=1000 ppm (hepatocytic hypertrophy and other liver effects). Dose levels included 3-10,000 ppm in the diet.	N/A	Guideline
Teratology - rabbit; Rohm & Haas/ 83R-216; 10/31/84	Technical 84.5% pure	266097	Levels tested by gavage in New Zealand White strain-0, 10, 31.6, 100, 215, 464 and 700 mg/kg/day Maternal NOEL = 100 mg/kg/day Maternal LEL = 215 mg/kg/day (abortion, increased incidences of irregular feces & red urine). Fetotox NOEL = 100 mg/kg/day Fetotox LEL = 215 mg/kg/day (greater incidences of resorptions). Not a teratogen up to 215 mg/kg/day, the highest dose level at which term fetuses were available. Range-Finding study.	N/A	Supplementary
Teratology - rabbit; Rohm & Haas/ 83R-217; 11/15/84	Technical 90.4% pure	266098	Levels tested by gavage in New Zealand White strain-0, 20, 60 and 200 mg/kg/day. Maternal NOEL=20 mg/kg/day (reduced body wt gain). Fetoembryotox. NOEL=60 mg/kg/day (increased # resorptions/litter, # litters with >2 resorptions, and # litters totally resorbed; reduced Viability Ind.;reduced litter size). Not teratogenic up to highest dose level tested (200 mg/kg/day). A/D ratio = 0.33.	N/A	Minimum

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
2-4 Week feeding - dog; Rohm & Haas/#83R-078; 10/8/86	Technical 84.5% pure	266078	NOEL = 250 ppm, LOEL = 1000 ppm (reduction in food consumption and bodyweights). Male and female Beagle dogs. Range-finding study.	N/A	Supplementary
One year chronic feeding - dog; Rohm & Haas/ #84R-078; 10/15/86 10/15/86	Technical 91.4% pure	266088	NOEL = 100 ppm; LOEL = 400 ppm (hepatocellular hypertrophy and supporting clinical chemistry and organ weights). Male and female Beagle dogs.	N/A	Minimum
2-year chronic feeding/ oncogenicity - rat; Tegeris Labs, Laurel, MD #'s 85RC-61, 8342; 10/24/86	Technical 90.4% + 91.4% pure	266081	NOEL=50 ppm (2.5 mg/kg/day); LOEL= 200 ppm (10 mg/kg/day);(testicular atrophy). No oncogenic effects observed. Levels tested:0, 25-35-50, 100-140-200, 400-560-800 ppm (first dose 2 weeks, second dose 2 weeks, third dose remainder of time). Sprague-Dawley. MTD may not have been reached.	N/A	Guideline for chronic feeding; Supplementary for oncogenicity
2-Year chronic feeding/ Oncogenicity -mouse; Rohm & Haas; 84R-023; 10/17/86	Technical 90.4% pure	266090	NOEL=20 ppm; LOEL=100 ppm (slight increase in liver mixed function oxidase). At 500 ppm, centrilobular hepatocytic hypertrophy, Kupffer cell pigmentation, periportal punctate vacuolation, individual hepatocell. necrosis. Negative for oncogenicity, but MTD may not have been reached. CD-1 strain mice. Dose levels tested: 0, 20, 100 and 500 ppm in diet.	N/A	Guideline for chronic feeding; Supplementary for oncogenicity
Mutagenic-Dominant lethal-rats; Argus Research Labs #86RC-0054; 10/10/86	Technical 91.4% pure	266101	Did not induce dominant lethal mutations under conditions of study at dose levels up to 735 mg/kg. Needs positive control data to update CORE grade.	N/A	Unacceptable

080800

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenic-In Vitro Cytogenetics;Litton Bionetics, Kensington, MD; [illegible]; 1/85	Technical 91.9% pure	266099	Does not induce chromosomal aberrations either with or without metabolic activation under conditions of study up to 200 micrograms/ml.	N/A	Acceptable
Metabolism-mice; Rohm & Haas; 83R-175; 8/29/86	Technical 81.1% pure; 99.7% radiopure.	266102	Rapidly absorbed and excreted. Completely eliminated by 96 hours. Extensively metabolized prior to excretion. Metabolic patterns similar for both sexes. Disposition & metabolism after pulse admin. linear over dose range tested.	N/A	Acceptable
Metabolism-rats; Rohm & Haas; 83R-144; 8/29/86	Technical 81.1% pure	266103	Completely and rapidly absorbed, extensively metabolized, rapidly and completely excreted. Elimination from plasma biphasic; eliminated dose evenly divided between feces + urine. No tissue accum. by 96 hours. Some quant. differences between males and females. Pretreatment for 2 weeks with nonlabelled material had little effect on disposition and metabolism.	N/A	Acceptable
Metabolism - cats; Rohm & Haas (Spring House Res. Labs); 310-84-16; 6/22/84	Technical - purity not given, but was synthesized with 14-C.	072904	Extensively metabolized + excreted in urine + feces. 7 major metabolites isolated + identified. Recovery of radioactivity 97.2%. Highest amts. of radioactivity found in liver, kidneys, large and small intestines. No bioaccumula.	N/A	Acceptable
Dermal Absorption - rats [illegible]; 8/26/86	Analytical>99% pure	266104	Failure to perform analysis of application site skin and residue make it impossible to verify recovery.	N/A	Unacceptable

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
4-week dermal - rats; Rohm & Haas; 85R-240; [illegible]	40WP Formulation - 41.36% pure.	266080	Systemic NOEL=100 mg a.i./kg/day (HDT). NOEL for skin irritation = 10 mg a.i./kg/day.	N/A	Minimum

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
[redacted]					
PENDING	REGISTRATION	ACTION	INFORMATION	NOT	INCLUDED

[illegible] dog; [illegible] Haas Co.; #[illegible]; [illegible]. 7, 1984	RH-3866 Tech Sample #TD83-076 Lot # LSPL00-16/E 81.1% pure	072899 072900	Levels tested for beagles - 0,10, 200,800 and 1600 ppm. Systemic NOEL = 10 ppm Systemic LEL = 200 ppm (liver centrilobular or midzonal hepatocellular hypertrophy)		Minimum 004937
[illegible]-In Vivo; Cytogenetic in mice; Rohm and [illegible]; #448-0074; [illegible]	RH-3866 Lot # LSPL-001-6/E 81.1% pure	072901	The level of 650 mg/kg did not cause a significant increase in chromosomal aberrations in bone marrow cells sampled over the entire mitotic cycle.		Acceptable 004937
[illegible]; Rohm [illegible]; #[illegible]; 1/31/	RH-53866 Tech Lot # LAP-0298 90.4% pure	072901	No appreciable increase in the reversion to histidine prototrophy of four S. typhimurium strains at 75 to 7500 ug/plate with and without S-9 activation.		Acceptable 004937
[illegible] - rat; Rohm & Haas; [illegible] #448 117; Aug. [illegible], 1985	Tech 84.5% ai RH-3866		Levels tested in Sprague-Dawley strain-0,50,200 and 1000 ppm. Systemic NOEL = 50 ppm Systemic LEL = 200 ppm (increased absolute and relative liver wts. and increased centrilobular hepatic hypertrophy in males). Reproduction NOEL = 200 ppm Reproduction LEL = 1000 ppm (testicular, epididymal, and prostatic atrophy in P_2 males and in both generations, increased numbers of stillborns and decreased wt. gain in pups during lactation.)		Guideline 004936

080830

[illegible] - CHO/HGPRT Forward Mutation Assay; Rohm & Haas; #84R-046; 5/29/84	Tech RH-53,866 81.1% pure Lot # LSPL0016/E	072901	Negative with and without S-9 activation up to 175/ug/ml		Acceptable 004936
Acute oral LD_{50} - rat; Rohm & Haas; #84-063A; 7/19/84	Tech Lot #83159-5 91.9% pure	072896	LD_{50} = 1.60 gm/kg (male) LD_{50} = 2.29 gm/kg (female)	III	Minimum 004936
Acute oral LD_{50} - mice; Rohm & Haas; #84R-0153; 6/3/84	Tech Lot #83159-5 91.9% pure	072896	LD_{50} > 4.42 gm/kg	III	Supplementary 004936
Acute dermal LD_{50} -rabbit; Rohm & Haas; #84R-134A; 7/30/84	Tech Lot #83159-5	072896	LD_{50} > 5 gm/kg	III	Minimum 004936
Primary dermal irr.-rabbit; Rohm & Haas; #84R-134A; 6/3/84	Tech Lot #83159-5 91.9% pure	072896	Non-irr.	IV	Minimum 004936
Primary eye irr.-rabbit; Rohm & Haas; #84R134A; 6/3/84	Tech, 91.9% pure	072896	Severe eye irritant		Minimum 004936

080800

[illegible] rat; [illegible] #83R-066; [illegible]	Tech 81.1 % pure Lot # LSPL00161E	072897 072898	Levels tested in Charles River COBS-CD(SD)BR strain-0,5,15,50,150,500, 1,500,5000 and 15,000 ppm for two wks. then to 7,21,70,210,700,2100, 7000 and 21000 ppm for weeks three and four, then to 10,30,100,300, 1000,3000, 10,000 and 30,000 ppm for weeks 5-13. NOEL = 1000 ppm LEL = 3000 ppm (increased liver and kidney wts.; hepatocellular necrosis and hepatocellular hypertrophy; pigmented convoluted tubules of kidney and vacuolated adrenal cortex.		Minimum 004936
[illegible]atology - rat; Rohm [illegible]; #83R-024; 6/22/[illegible]	Tech Lot # LSPL83-0017E 84.7% pure	072901	Level tested by gavage in Sprague-Dawley [CrL:CD-(SD)BR] strain - 0, 31.26,93.77,312.58 and 468.87 mg/kg on gestation days 6-15. Maternal NOEL = 312.6 mg/kg/day Maternal LEL = 468.9 mg/kg/day (decreased body wt., salivation, alopecia, desquamation and red exudate around the mouth. Developmental NOEL = 31.3 mg/kg Developmental LEL = 93.8 mg/kg (increased resorption, decreased viability) A/D ratio = 312.6/31.3 = 9		Minimum 004936

[illegible] 84R-077B; 7/16/84	RH-53,866 Tech (24.0% ai)		50 [illegible] (0.98 1.60) gm/kg (F)	—	004936

PENDING REGISTRATION ACTION NOT INCLUDED

[redacted]

[illegible], eye irr.-rabbit; [illegible] Haas; #84R-082A; [illegible]/84	40WP 39.5% ai Lot #EG-0809-1	072896	Moderately irritating to the eye.	III	Supplementary 004936

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[illegible]	Sample #84-39				
Primary dermal irrit - rabbit; Rohm & Haas; #84R-082A; 7/16/84	40 WP 39.5% ai Lot #EG-0809-1	072896	Mild irritant	IV	Minimum 004936
[illegible] dermal LD_{50}-rabbit; Rohm & Haas; #84R-082-[illegible] and 84R-082B; 7/16/84	40 WP 39.5% ai Lot #EG-0809	072896	LD_{50} > 5 gm/kg		Minimum 004936
[illegible] oral LD_{50} - rat; Rohm & Haas; #84R-082A and 84R-082B; 7/16/84	40 WP 39.5% ai Lot #EG-0809-1	072896	LD_{50} = 1.87 gm/kg (M) LD_{50} = 2.09 gm/kg (F) PENDING REGISTRATION ACTION NOT INCLUDED	III	Minimum 004936
[redacted]					
[illegible] feeding/onco - rat; Rohm & Haas; #84R-[illegible]A; 7/16/84	RH-53866	073522 073805 073806 073807	Interim report 3/87		004936
[illegible] feeding-dog; Rohm & Haas; #84R-078; [illegible], 1985	RH-53866	073805	Interim report Levels tested - 0,10,100,400 and 1600 ppm		004936

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(21)

[redacted]

Miscellaneous

3/21/80

Cleared Fungicide

STUDY TITLE

RALLY® Fungicide
Response to TB Peer Review on the Adequacy of HDT for Oncogenicity Studies in Rats and Mice

DATA REQUIREMENT

Guideline No. 83-5

AUTHOR

P. K. Chan, Ph.D., D.A.B.T.

STUDY COMPLETED ON

March 21, 1988

PERFORMING LABORATORY

Toxicology Department
Rohm and Haas Company
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LABORATORY PROJECT ID

88R-051

MYCLOBUTANIL 128857

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124-144

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RALLY® Fungicide
Response to TB Peer Review on the Adequacy of HDT for Oncogenicity Studies in Rats and Mice
Report No. 88R-051

EXECUTIVE SUMMARY

I. Response to TB Peer Review of 2-year Rat Study

EPA MTD Concern

- Rat chronic study effects do not appear to be life-threatening.
- Depressed body weight gain (BWG) is insufficient since values returned to control values by the very end of the study.
- Predicted MTD from 90-day study was 3000 ppm.

Rohm and Haas Re-evaluation

A. Actual Data from the 2-year chronic/oncogenicity study

Testicular Atrophy

- Incidence of testicular atrophy was 3x control at 200 ppm and 6x control at 800 ppm.
- Atrophy is indicative of specific dose related target organ stress sufficient to induce oncogenic effects if biologically possible.

Body Weight Gain (BWG)

- Male BWG depression reached -7% in the first year and -12% in the second year (Figure 1, Table 1).
- Female BWG depression reached -5% in the first year and -17% in the second year (Figure 2, Table 2).
- Convergence of high dose BWG with values by the very end of the study is an artifact caused by accelerated control decline at the most prominent aging period (Figure 3 and 4).

Liver Effects

- Liver weight was significantly increased in females (13 - 20 %; $p<0.05$) at 800 ppm at 3 and 6 months.
- MFO was increased in males and females at 800 ppm in all periods evaluated. Female values were 147-178% of control.

145

- These effects may not be life-threatening per se, but indicate additional target organ stress.

B. Predicted MTD from 90-day Feeding Study

- 90-day BWG depression at 3000 ppm reached -19% in males and -16% in females and was decreasing (Figure 1 and 2; Table 3).
- Extrapolating to 2 years, BWG depression would have continued to a maximum of -25% for males and -30% for females (Figure 1 and 2).
- Effects seen at 3000 ppm were: BWG depression, liver hypertropy, cell necrosis, increased liver weight and MFO induction, increased kidney weight, renal toxicity (BUN; pigmentation of the renal tubular epithelium), increased serum cholesterol, and adrenal cortex vacuolization.
- At 1000 ppm BWG depression reached a reasonable - 10% as early as 6 weeks (Figure 1 and 2) as required by the new MTD criteria.
- The dose chosen was 800 ppm.

II. Response to EPA Peer Review of 2-year Mouse Study

EPA's MTD Concerns

- HDT was inadequate as MTD in female mice.
- Predicted MTD by TB was 3000 ppm.

Rohm and Haas Re-evaluation for Females

1. Actual data from the 2-year feeding study:
   - BWG depression was consistently at -10 to -15% % at the HDT (500 ppm) during the first 90 days; and averaging -5 to -10% through out the rest of the study (Table 4; Figure 5).
   - Liver toxicity at 500 ppm as MTD criteria included:
     - Increased liver weight (12 - 13%) at 3 months
     - MFO induction at all periods evaluated (3, 6, and 12 months)
     - SGPT increase (60%) at 3 months
     - Hepatocellular vacuolization at terminal sacrifice
     - Hepatocellular alterations of all types at terminal sacrifice

146

RALLY® Fungicide
Response to TB Peer Review on the Adequacy of HDT for Oncogenicity Studies in Rats and Mice
Report No. 88R-051

2. Predicted MTD from 90-day feeding study:

   - Decreased BWG at 500 ppm in the 2-year study was not seen at 1000 ppm in the 90 day study. This was due to age differences; older animals were used in the 90-day feeding study (Table 4, 5).

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